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COMPARATIVE WEED INVESTIGATIONS IN WHEAT AND MAIZE CROPS CULTIVATED TRADITIONALLY AND TREATED WITH WEEDICIDES I. CHANGES IN THE WEED VEGETATION OF WHEAT CROPS

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Introduction

Coenological recordings in 1961 proved that as a result of the up-to-date large-scale agricultural techniques applied in the socialistic reorganization of agriculture very considerable changes took place in the weed vegetation as compared to the recordings in 1947–1953. Their cover decreased considerably in every case, and particularly for weeds perennating in the soil (G), while at the same time the number of species increased in the majority of cases (FEKETE, 1963); that is to say, the weed conditions at present differ completely from those at the time of the national weed recordings.

After this favourable change was established in the weed vegetation of wheat and maize crops under traditional agricultural conditions, it was later considered necessary to extend the investigations to the areas treated with chemical weed-killers as the development of weeds under these agrotechnical conditions was considerably influenced by the increasingly general application of the various herbicides (2,4–D, aminotriazines). It was therefore considered justified to establish the roles of the developed large-scale agrotechnology and the various systematically applied chemical weed-killers in the change in weed vegetation, as compared to the conditions in 1949–1950.

In this way, from 1963 a study was made on fixed investigation sites with regard to the qualitative and quantitative conditions of the weed vegetation of (i) wheat crops; untreated cultures and those treated with Dikonirt or under the after-effect of Simazin and (ii) maize crops; cultured traditionally, sprayed first with Hungazin PK, treated with HPK for several years and for two-three years under the after-effect of being sprayed with HPK.

To establish the changes in the weeds during the last decade, the results of the investigations were compared with the data of ÚJVÁROSI (1950, 1961), for the same places.

I made my paper in the spring of 1964 from the coenological results of the first investigations (1963) and from those concerning the weedicides of the soil, but it could not be published. In 1967, I elaborated the manuscript again, to meet the requirements of a doctoral dissertation, completing it with the results of my investigations on the weed-seed content of the soils. The present paper is containing the part of the old manuscript, resp. of the doctoral disser-

tation elaborated out of that, concerning the weed vegetation of wheat crops. The work was carried out in the Botanical Research Institute of the Hungarian Academy of Sciences at Vácrátót.

Places and methods of investigations

Except for a farm at Enying, the investigation sites were chosen so as to belong to the areas examined in detail on the occasion of the national weed recording. The investigations were carried out on state Farms at Fehérgyarmat, Mezőnagymihály, Mezőhék, Enying, Lábod and Kaposvár. In some of these farms several units were recorded. From among the national weed recording data those of Mezőkövesd were used for a comparison with the weed conditions at Mezőnagymihály, those of Mezőtúr with those of Mezőhék, and those of Kadarkút with Lábod. The areas recorded in 1949—1950 belong at present to the farms mentioned above. In the case of the State Farms and Fehérgyarmat and Kaposvár there has been no change in name.

The wheat received the necessary agrotechnical treatment on every farm and in a suitable quality during the preparation for the sowing and later too. The wheat treated with Dikonirt was sprayed by the farms May 5 to 10, with doses of 1.2—1.3 kg per cadastral yoke. The wheat areas examined for the "after-effect" were sprayed with 7 kg Simazin per cad. yoke at Mezőnagymihály in the spring of 1961. In one of the areas investigated at Enying 5 kg Simazin per cad. yoke was applied in the autumn of 1960, and in another area in the spring of 1961. The herbicides were administered by aeroplane in all cases, except for the single area of autumn spraying at Enying, where a land machine was used. The recording was carried out with the coenological method of BALÁZS (1944). In every investigation site separate recording series were made for the traditional wheat, that with Dikonirt, and that with the Simazin after-effect. Ten recordings were performed for each treatment on every site, and twenty in the case of areas larger than 300 cad. yokes. The recording sites were distributed proportionately to the number of wheat-fields, with in general two recordings per field. (As the farm units were specialized and put in blocks, the fields investigated were everywhere localized beside or close to one another.) Both the earlier recording and those in 1963 were carried out in June. The recording series in every investigation site were evaluated separately, according to the treatment. The results cannot be reported here owing to the limited size of this publication, however desirable it would be from the point of view of the protection against weeds to give information about the mass relations of at least the more important species in every field separately. These data are contained, however, in a doctoral dissertation (FEKETE, 1967, defended in 1972). In the framework of the present publication it is possible only to list those weed species (Table 1), which cover an area of larger than 1% on an overall average for the treatment in each of the different investigation sites.

Table 1. Major weed species of wheat crops and their covering percentages ofter various treatments, on the basis of the summarized data for the sites investigated

Treatment	Traditional	Traditional	With Dikonirt	Simazin after-effect
Year of recording	1950	1963	1963	1963

The recordings were carried out in accordance with the investigations of the Geological and Agrochemical Research Institute of the Hungarian Academy of Sciences, at Fehérgyarmat in soil of clayey adobe, and to a smaller extent of sandy adobe. On the State Farm of Mezőnagymihály the recording of wheat took place in strongly bound adobe and clayey adobe soils, at Mezőhék and Enying in open country adobe, at Lábod in sandy adobe and adobe, and at Kaposvár in harder and lighter adobe soils.

It is characteristic of the precipitation conditions that at the time of the national recording (1949—1950) in some of the places investigated there was much drier weather than the 40 years' average.

In 1963, the precipitation totals corresponded to the many-year averages characteristic of the areas, but at Mezőhék it was higher than this. At Lábod and Kaposvár there was only very little rain in April 12 and 13 mm respectively.

Results of the investigations and their evaluation

The most important weeds of wheat crops and the number of species and cover values for the individual life forms (ÚJVÁROSI, 1952) are given in Tables 1 and 2, on the basis of the overall data of the investigation sites and treat-

Table 2. Numbers and covering percentages of the weed species belonging to the individual life forms, as averages of the areas investigated, according to the results of the recordings in 1949—1950 and 1963.

Treatment	Traditional		Traditional		With Dikonirt		Simazin after-effect	
Number of species and cover percentage	1	2	1	2	1	2	1	2
Life forms:								
T_1	16	0.81	15	0.71	5	0.05	2	0.02
T_2	31	11.98	25	1.58	15	1.28	5	0.14
T_3	11	5.15	13	2.30	8	2.52	6	1.33
T_4	30	7.32	42	7.63	37	3.50	28	16.25
Total T	88	25.26	95	12.22	65	7.35	41	17.74
HT	1	0.01	1	0.01	2	0.01		
H_3	5	0.19	4	0.01	3	0.01	3	0.03
H_5			3	0.10	3	0.01		
Total H	5	0.24	8	0.11	8	0.02	3	0.03
G_1	6	6.85	8	1.20	4	1.58	3	1.06
G_2	1	0.02			1	0.01		
G_3	9	10.40	7	5.25	7	4.29	4	9.63
G_4	3	0.03	2	0.02	2	0.01	1	0.01
Total G	19	17.35	17	6.47	14	5.89	8	10.70
Number and covering percentage of the total weed species	113	42.86	120	18.13	87	13.27	52	28.47

1 : Number of species
2 : Percentage of covering
T_1: Wintering one-year old weeds in early spring
T_2: Early-summer one-year old weeds shooting in autumn
T_3: Early-summer weeds shooting in spring
T_4: Late-summer one-year old weeds
H_3: Tap-rooted weeds
H_5: Weeds with inclined rhizome
G_1: Creeping bent grass (Couch-grass)
G_2: Tuber crops
G_3: Weeds with couch-grass-like roots

ments. The Tables contain the data of the weed recordings from 1949–1950, too, (Újvárosi, 1950; 1961) for purposes of comparison. These and Figs. 1–3 indicate the extent of the changes in the weeds of wheat crops since the national recordings, due partly to the traditional cultivation, and partly to chem-

ical weed-killers, and the group or groups of life-forms among these which were most affected by these changes.

1. Effect of the agrotechnology in large-scale farming of the development og weeds in wheat crops

In the wheat crops of the farms chosen as the investigation sites, 113 weed species occurred in the recordings of 1949–1950, with an average cover of 42.86%. In 1963, according to the overall data, 120 species were found in the traditionally cultivated wheat crops, with an average cover of 18.81%. During 13 years, therefore, the weed cover of the wheat crops decreased by more than half as a result of the more up-to-date agricultural techniques in the large-scale farming (Table 2).

Comparing the weeds according to life forms, the following changes are found:

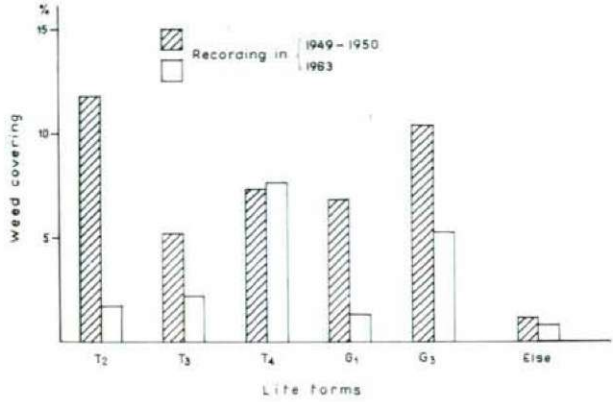

Fig. 1. Influence of the agrotechnology in large-scale farming on the formation of weeds in wheat crops, according to life forms.

As indicated in Fig. 1 and Table 2, the early summer weeds shooting in autumn (T_2) have completely lost their earlier importance, for their cover is reduced to nearly a tenth of the old value (from 11.98 to 1.58%) and the number of species too is appreciably lower than before (25 instead of 31). Some spiked weeds of frequent occurrence, belonging to group T_2, such as *Centaurea cyanus, Consolida orientalis* and *Vicia* species, have fallen completely into the background (Table 1).

The early summer weeds shooting in spring (T_2) have similarly decreased in quantity, to be about half the old cover (from 5.15 to 2.3%), primarily as a result of the reduction of Sinapis arvensis and Scleranthus annuus.

For those from the late summer (T_4), however, it is observed that their cover has risen somewhat compared with those in 1949–1950 (from 7.32 to 7.63%). The explanation of the unchanged, or slightly increased amount of those belonging to group T_4 is mainly that the winter of 1962/63 was extremely hard, and unfavourable to the crops in standing the winter; in addition the Italian wheat species known to be much more sensitive to cold were also sporadically recorded. These crops were therefore much thinner than the others, and this weed group could easily prevail in them. Although the amount

of those from the late summer remained essentially unchanged, the cover values of a large proportion of the weed species belonging here are changed. For example, *Ambrosia elatior Polygonum aviculare* and *P. convolvulus* are now to be found to a considerably lower extent. At the same time, some species have counteracted the decrease of the above species with their rapid propagation. The number of species of life form T_4 has also increased, similarly to the cover, from 20 to 42. A lesser pushing forward of the thermophilous late summer weeds seems to support the earlier findings (UBRIZSY, 1954; TIMÁR–UBRIZSY, 1957).

Wheat overgrown with perennial radiciform couch-grass (G_3) occurred with 10.4% in the investigation sites in 1949–1950. It is noteworthy that the cover of the weed group decreased in the 13 years to 5.25%, about half the old cover. Although they can still be found sporadically in considerable amount (e. g. at Fehérgyarmat), on the majority of farms they are on longer considerable. Similarly to the results of investigations in 1961 (FEKETE, 1963), the highest decrease in the group is for *Convolvulus arvensis,* which occurs nationally in the greatest quantity, but here its cover has fallen from 6.67 to 2.44% (Table 1). A similar change, but to a still higher degree, may be observed in the case of couch-grasses (G_1). The cover of this weed group in 1949–1950 was 6.85% in the overall average of the investigated sites. The present cover of couch-grasses, however, is only 1.2% (Table 2). Of the weeds belonging here, *Equisetum arvense* has decreased significantly, in spite of its still being considerable at Lábod.

Compared to the weed groups discussed so far, weeds belonging to other life forms are negligible in both investigations, and are therefore not discussed in detail.

2. Effect of Dikonirt spraying on the formation of weeds in wheat crops

Dikonirt-treated wheat-fields were recorded at Fehérgyarmat, Mezőnagymihály, Mezőhék and Lábod.

Comparing the wheat crops of the present traditional cultivation with Dikonirt-sprayed ones, an additional 27% decrease occurred in the weed cover as a result of the herbicide (Table 2). As the average for the four farms, the weed cover of the wheat with Dikonirt is 13.27%, which can be regarded as still fairly high after the chemical weed control.

Investigation of the effect of chemical treatment upon the covers of the weeds belonging to the individual life forms leads to the following findings (Fig. 2).

The cover of groups T_2 and T_3 has hardly changed in essence as a result of Dikonirt. In the case of these two life forms, therefore, the major decrease compared to the cover in 1950 was a result not of Dikonirt but primarily of the better agrotechnology applied. The use of herbicides proved effectual to various extents for every weed species except two. The somewhat larger cover of T_3 was caused by the propagation of *Fumaria* in the Dikonirt wheat at Mezőnagymihály and Mezőhék. *Galium aparine,* belonging to T_2, similarly increased.

An appreciable decrease as a result of Dikonirt can be demonstrated in the covers of the one-year old weeds from *late summer* (T_4): 3.50% on average

for the sites investigated in the chemically treated wheat, and 7.63% in that traditionally cultivated. The most sensitive response to the Dikonirt spraying was given by *Stachys annua* and *Ambrosia elatior,* but some effect can be dem-

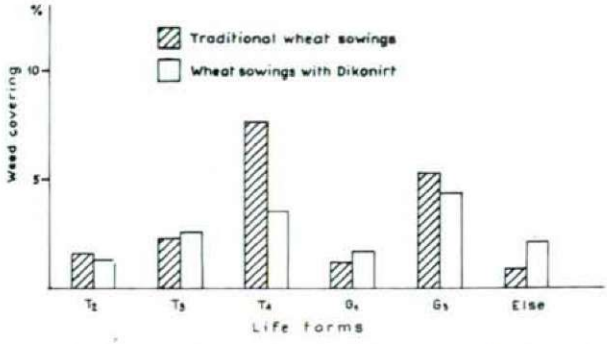

Fig. 2. Influence of Dikonirt spraying on the formation of weeds in wheat crops, according to life forms.

onstrated on nearly every T_4 weed species. The larger amount of *Chenopodium* can be explained by its propagation among the Italian wheat at Mezőhék. It is obvious from the Figure and Tables that the weed-killing effect of Dikonirt was the most efficient in the case of this life form.

It is known that the late summer weeds (T_4) shoot en masse in densely growing wheat in May and June, and even earlier in thinly growing wheat.

Spraying of wheat with Dikonirt is usually carried out from the middle of April till the middle of May; on the farms investigated for instance, it was performed everywhere between May 5 and 10. As the effect of Dikonirt spraying lasts for about 4–5 weeks, the influence of the herbicide extends to the period when the late summer weeds sprout; these therefore come into contact with the weedicide in just that stage when they are biologically the most sensitive to it. In contrast to earlier findings (UBRIZSY, 1958a,b), this is the explanation for the very sensitive reaction of the late summer weeds (T_4) to Dikonirt spraying. At the same time, the cause of the low effect of herbicides on the life forms T_2 and T_3 may be that at the time of their being sprayed some of the species belonging to these weed groups are already over the period of being sensitive to herbicides.

Of the perennial weeds wintering in the soil, the cover of couch-grasses (G_1) was not decreased by Dikonirt. In the case of the radiciform couch-grasses (G_3), however, a decrease of about 20% could be observed (from 5.25 to 4.29%). It is true that *Convolvulus arvensis* has a somewhat larger cover value among wheats with Dikonirt, but the explanation of this may presumably be that the cover there was originally larger than in untreated crops (Table 1).

3. Development of weeds in wheat crops exposed to Simazin after-effect

Wheat crops with Simazin after-effect were recorded at Mezőnagymihály and Enying. At Mezőnagymihály, the crop-field was treated with 7 kg Simazin per cad. yoke in the spring of 1961. At Enying, some of the fields investigated were sprayed with 5 kg Simazin per cad. yoke in the autumn of 1960, and

the rest in the spring of 1961. In these two areas, the extents of the damage and the weed cover of the wheat were different, and thus separate recording series were made for them.

At Mezőnagymihály, and in the field sprayed in the autumn of 1961 at Enying, the crop began to turn yellow in April and May of 1963 and, owing to the after-effect, about 15–20% of the wheat perished. Empty spots developed sporadically, but the whole crop became thinner, and the place of the destroyed crop was partly occupied by weeds, correspondingly, in contrast to the 18.81% weed cover of the traditional wheat crops, the weed pollution of the fields under after-effect increased to 28.47% (Table 2).

In the wheat crops with Simazin after-effect, two weed groups in particular were found in large numbers: the one-year old weeds from the late summer (T_4) and the perennial radiciform couch-grasses wintering in the soil (Fig. 3).

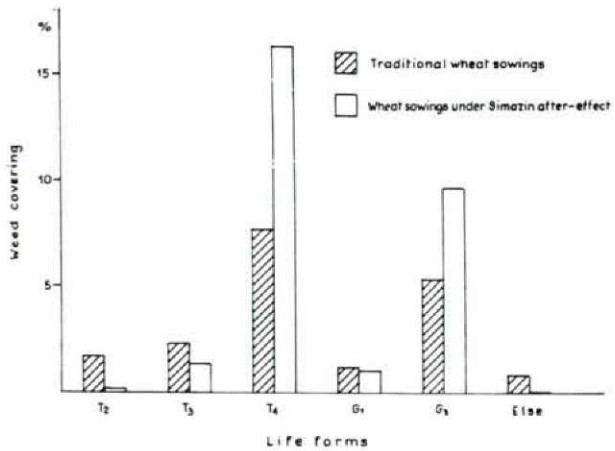

Fig. 3. Development of weeds in the wheat crops with Simazin after-effect, according to life forms.

The average cover of the late summer weeds (T_4) in the sites investigated proved to be 16.25%, their presence being more than twice as frequent as in the crops cultivated in the traditional way. Life from T_4 occurred in extremely high numbers, with 23.29%, at Mezőnagymihály where *Echinochloa crus-galli* propagated itself to an incredible degree (16.66%).

According to the investigations of Újvárosi, (1951), densely growing cereals, including wheat, exert an effect on the development of weeds shooting among them. Here, however, the crop became thin due to the after-effect and the weed-suppressing influence could no longer prevail in a suitable way from the beginning of May, this resulting in the mass propagation of the late summer weeds.

Of the perennials, the advance of the radiciform couch-grasses (G_3) could be observed, their average cover being 9.63% in the wheat with Simazin after-effect, compared to 5.25% in the non-chemically treated fields. In the groups, mainly *Rubus caesius* prevailed with its large mass.

The Simazin after-effect, and the consequent overgrowth with weed, manifested itself in the crop results, too, for in the fields affected 15–25% less wheat grew than in the untreated ones.

Summary

In 1963, weed recordings were carried out in fields the weed conditions of which had been elaborated in detail by ÚJVÁROSI in 1949–1950. Traditional wheat crops and those treated with Dikonirt or under Simazin after-effect were investigated to establish the roles of the individual factors in the development of the weeds.

The recording results can be summed up as follows:

1. The agrotechnology of large-scale farming makes its effect felt in a definitely positive way on weeds in wheat crops. In the traditional cultivation the weed cover of the wheat crops investigated decreased 56% as compared to those in 1949–1950, that is to less than half the previous cover, merely as a result of more up-to-date agrotechnical processes. Within this favourable change in the total cover the differences for the single life forms are of various extents. The amount of one-vear old weeds (T) decreased to be about half year the old value. The early summer weeds (T_2) shooting in autumn, and occurring in large numbers in the old wheat crops, lost their earlier importance both as to the number of species and to the cover. The decrease in early summer weeds shooting in spring (T_3) was 50% or so. The number of species and the cover of those from late summer (T_4), however, somewhat increased. The increase in the latter can be explained mainly by their propagation among the thinly growing Italian wheats where this weed group could easily prevail. The weeds wintering in the soil (G) were forced back to one-third of the control value during the 13 years. The decrease in couch-grasses (G_1) was higher (80%) than that in the radiciform couch-grasses (47%). As in the earlier investigations the weed-decreasing the effect of the agrotechnology of large-scale farming is therefore definitely proved.

2. Spraying with Dikonirt resulted in a further 27% decrease in the total weed-cover of the wheat crops. The reactions of the individual weed groups to the hormone-based herbicides, however, were different. Of the one-year olds, the cover of T_2 hardly changed as a result of 2,4–D. The cover of group T_3 grew a little, but there was a very considerable decrease (50%) in that of T_4. The herbicide effect of Dikonirt, therefore proved to be most effective with this life form. Of those wintering in the soil, group G_1 was not affected at all by Dikonirt; a 20% decrease was induced in the amount of G_3, however.

3. In areas treated with Simazin, about 15–20% of the wheat perished in the third year after being sprayed, due to the after-effect. This resulted in 60% more weed cover as compared to the control. In crops becoming thinner as a consequence of the after-effect, particularly some members of weed groups T_4 and G_3 propagated themselves in large quantities. As a result of all these factors the crop of those areas decreased by 20–25%. The data from the investigations therefore suggest the more careful agricultural administration of the areas under Simazin after-effect.

References

BALÁZS, F. (1944): A növénycönológiai felvételek készítésének újabb módjai (New methods of carrying out plant coenological recordings). — Bot. Közl. *41*, 18—33.

FEKETE, R. (1963): Változások a szántóföldi gyomnövényzetben (Changes in field-weed vegetation). — Hung. Acad. of Sci. Publ. of the Dept. of Agr. Sci. *22*, 377—393.

FEKETE, R. (1964): Összehasonlító gyomvizsgálatok hagyományos művelésű és vegyszeresen kezelt búza- és kukoricavetésekben (Comparative weed-investigations in wheat and maize crops cultivated traditionally and treated with chemical herbicides. Manuscript, elaborated in the form of Doct. Diss., 1967; defence of the thesis 1972).

I'SÓ, J. (1962): Utóhatás-kísérlet őszi búzában klóraminotriazinokkal kezelt kukorica után. Kukoricatermesztési kísérletek 1958—1960 (After-effect experiment in winter wheat, after maize treated with chloraminotriazines. Experiments in maize growing. 1958—1960). 363—369.

I'SÓ, J. (1963): A Hungazin, Simazin, Atrazin utóhatásáról (The after-effects of Hungazin, Simazin and Atrazin). — Magyar Mezőg. *12*, 10—11.

TIMÁR, L.—UBRIZSY, G. (1957): Die Ackerunkräuter Ungarns mit besonderer Rücksicht auf die chemische Unkrautbekämpfung. — Acta Agron. Acad. Sci. Hung. *7*, 123—150.

UBRIZSY, G. (1954): Vizsgálatok őszi búzavetés agrofitocönózisában (Investigations into the agrophytocoenosis of winter wheat). — Növénytermesztés *4*, 281—300.

UBRIZSY, G. (1958a): Cönológiai kutatások agrárterületek gyomtársulásain, különös tekintettel a vegyszeres gyomirtás flóraátalakító hatására (Coenological investigations of the weed coenoses of agricultural areas, with particular regard to the flora-transformative effects of chemical weed-killers). — Biol. Group, Hung. Acad. of Sci. *2*, 65—78.

UBRIZSY, G. (1958b): Vegyszeres gyomirtás gabonavetésekben (Chemical weed-killing in corn crops). — Dept. of Agr. Sci., Hung. Acad. of Sci. *14*, 339—418.

UBRIZSY, G. (1962): Vegyszeres gyomirtás (Chemical weed-killing). — Agr. Publ. House, Budapest.

ÚJVÁROSI, M. (1950): Hol milyen gyomok ellen védekezzünk? (Where and against which weeds should we protect?) — Yearbook of the Agric. Res. Inst. in Debrecen, *1*, 27—105.

ÚJVÁROSI, M. (1951): Szántóföldi kísérletek a különböző gabonavetések gyomirtó hatásának vizsgálatára (Field experiments to investigate the weed-killing effects of various field crops). — Biol. and Agr. Dept. of the Hung. Acad. of Sci. *2*, 145—149.

ÚJVÁROSI, M. (1952): Szántóföldjeink gyomnövényfajai és életformaanalízisük (Weed species on cultivated land in Hungary and analysis of their life forms). — Növénytermesztés *1*, 27—50.

ÚJVÁROSI, M. (1957): Gyomnövények, gyomirtás (Weed plants and weed-killing). — Agricult. Publishing House. Budapest.

ÚJVÁROSI, M. (1961): A búzavetések gyomnövényzete. Búzatermesztési kísérletek 1952—1956 (Weeds in wheat crops. Experiments in wheat-growing, 1952—1956). — Publ. House of the Hung. Acad. of Sci. Budapest.

ÚJVÁROSI, M. (1966): A gyomnövényzet változása a szántóföldeken az elmúlt évtizedben (Changes in ploughland weeds in the past decade). — Agr. Dept., Hung. Acad. of Sci. *25*, 275—289.

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THE PRESENT STATE OF ULTRASTRUCTURAL RESEARCH INTO FOSSIL SPOROMORPHS

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Introduction

After the pioneering studies by EHRLICH and HALL (1959), modern research into the ultrastructures of fossil sporomorphs began with the work of PETTITT and CHALONER (1964) and then PETTITT (1966). The essential advance in these latter two publications was that the objects of transmission electron-microscope (TEM) studies were predetermined. Later, KEDVES and PÁRDUTZ (1970a,b) strongly urged the need for light-microscopic documentation to be given on samples intended for examination, before the preparation of ultrathin sections, in order that it be possible to follow any changes in terminology even afterwards. The TEM examination of important sporomorphs is generally carried out with the following aims:

1. The elucidation of the wall-structure of the fossill sporomorphs, particularly in the pore-wall region where light-microscopic examinations have frequently led to contradictory results.

2. The use of ultrastructural data in taxonomic descriptions, or the supplementation or necessary revision of the earlier descriptions.

3. For a more precise establishment of the botanical relationships, together with the light-microscopic morphology.

4. To draw evolutionary conclusions on the basis of the ultrastructures between sporomorphs of various geological ages and belonging to various types.

Numerous such partial results have already been or are now being published. The aim of the present paper is to collate and systematize the available data, and to extract the most essential correlations from these in the hope that these will give new starting points for future studies.

The literature data available in the history of the development of the flora are listed in main groups according to the sporomorphs. After the names of the individual species or form-species comes the geological age, and then in brackets the number of the literature reference where the detailed descriptions can be found.

At present ultrastructural results are known for the following listed taxons:

I. *Isosporous Pteridophyta* spores

Psilopsida

1. *Archaeotriletes* sp., U. Devonian (15).

Tmesopsida

2. *Microfoveolatosporis pseudodentatus* W. KR. 1959, L. Eocene (9).
   Pteropsida
   Leptosporangiatae
   Filicales
3. *Leiotriletes adriennis* (R. POT. and GELL. 1933) W. KR. 1959 asp. *triplanoid* KDS. 1961, L. Eocene (9).
4. *Appendicisporites tricuspidatus* WEYL. and GREIF. 1953, U. Cretaceous (9).
5. *Toroisporis (Toroisporis) eocenicus* KDS. 1966, L. Eocene (9).

II. *Heterosporous Pteridophyta* spores
   Lycopsida
   Lepidodendrales

6. *Laevigatisporites cf. glabratus* (ZERNDT) R. POT. and KREMP 1954, VIRGILLIAN (15).
   Pteropsida
   Primofilices
   Archaeopteridales
7. *Archaeopteris* cf. *jacksoni* DAWSON, Devonian (15).
   Hydropterides
8. *Azolla* cf. *aspera* DOROFEEV (1963), L. Pliocene (10).
9. *Azolla nana* DOROFEEV 1959, L. Miocene (10).
10. *Azolla tomentosa* NIKITIN ex DOROFEEV 1955 (10).
11. *Azolla teschiana* FLORSCHÜTZ 1945, L. Eocene – Palaeocene (10).
12. *Azolla tegeliensis* FLORSCHÜTZ 1938, L. Pleistocene (11).
13. *Salvinia cerebrata* DOROFEEV 1955, Pliocene/Pleistocene (12).
14. *Salvinia rhenana* KEMPF 1971, Pliocene/Pleistocene (12).

III. *Gymnospermatophyta* sporomorphs
   Pteridospermophytina
   Pteridospermopsida

15. *Schopfipollenites* sp., Virgillian (15).
16. *Trigonocarpus* sp., Virgillian (15).
17. *Cystosporites giganteus* (ZERNDT) SCHOPF 1938, L. Carboniferous (15).
18. *Didymosporites scotti* CHALONER, L. Carboniferous (15).
   Coniferophytina
   Cordaitopsida
19. *Florinites* sp., – (15).
   Coniferopsida
20. Cf. *Araucariacites* v. *Granulatisporites* fsp., U. Cretaceous (9).
21. *Spheripollenites scabratus* COUPER 1958, Jurassic (9).
22. *Classopollis* sp., *Cheirolepis muensteri*, Rhaetic (16).
23. *Classoidites glandis* AMEROM 1965, U. Cretaceous (9).
24. *Wodehouseia spinata* STANLEY 1961, U. Cretaceous (13).

IV. *Angiospermatophyta* pollen grains
   Dicotyledonopsida
   Brevaxones
   Normapolles

25. *Atlantopollis reticulatus* W. KR. 1967, U. Cretaceous (2, 3).
26. *Complexiopollis praeatumescens* W. KR. 1959 emend. HEGEDŰS, KEDVES and PÁRDUTZ 1972, U. Cretaceous (2, 3).
27. *Trudopollis mechanicus* PF. 1953, U. Cretaceous (3).

28. *Oculopollis zaklinskaiae* GÓCZÁN 1964, U. Cretaceous (2).
29. *Hungaropollis* fsp.$_1$, U. Cretaceous (2).
30. *Hungaropollis* fsp.$_2$, U. Cretaceous (2).
31. *Hungaropollis* fsp.$_3$, U. Cretaceous (2).
32. *Interporopollenites endotriangulus* HEGEDŰS, KEDVES and PÁRDUTZ 1972, U. Cretaceous (2).
33. *Vacuopollis orthopyramis* PF. 1953, U. Cretaceous (3).
34. *Basopollis basalis* (PF. 1953a) PF. 1953a, L. Eocene (7).
35. *Pompeckjoidaepollenites subhercynicus* (PF. 1953b) emend. W. KR. 1967, L. Eocene (6).
36. *Nudopollis terminalis* (PF. and TH. 1953) PF. 1953b subfsp. *hastaformis* PF. and TH. 1953, L. Eocene (6).
37. *Plicapollis pseudoexcelsus* (W. KR. 1958a) W. KR. 1961d subfsp. *turgidus* PF. 1953a, L. Eocene (6).
38. *Interpollis velum* W. KR. 1961d, L. Eocene (6).

Postnormapolles

39. *Triporopollenites robustus* PF. 1953a., L. Eocene (6).
40. *Subtriporopollenites constans* PF. 1953a subfsp. magnus W. KR. 1961d, L. Eocene (6).
41. *Intratriporopollenites microreticulatus* Mai 1961, L. Eocene (6).
42. *Compositoipollenites rhizophorus* (R. POT. 1934b) R. POT. 1960 subfsp. *burghasungensis* MÜRR. and PF. 1953, L. Eocene (6).
43. *Diporites iszkaszentgyörgyi* KDS. 1965, TEM diagnosis: KEDVES and PÁRDUTZ 1972a, L. Eocene (7).
44. *Teixeraipollenites globosus* PÁRDUTZ, JUHÁSZ, DINIZ and KEDVES 1972, U. Cretaceous (14).

Longaxones

45. *Transdanubiaepollenites magnus* KEDVES and PÁRDUTZ 1972a, L Eocene (7).
46. *Tricolporopollenites sooi* KEDVES and PÁRDUTZ 1972 a, L. Eocene (7).
47. *Tricolporopollenites miniverrucatus* ROCHE 1968, L. Eocene (7).
48. *Tricolporopollenites kruschi* (R. POT. 1931) TH. and PF. 1953 subfsp. *accessorius* (R. POT. 1934) TH. and PF. 1953, L. Eocene (7).
49. *Tricolporopollenites cingulum* (R. POT. 1934) TH. and PF. 1953 subfsp. *pusillus* (R. POT. 1934) TH. and PF. 1953, L. Eocene (7).
50. *Tricolporopollenites margaritatus* (R. POT. 1931a) TH. and PF. 1953 f. *medius* PF. and TH. 1953, L. Eocene (6).
51. *Tricolporopollenites* cf. *microreticulatus* PF. and Th. 1953, L. Eocene (7).
52. *Polycolpites viesenensis* W. KR. 1961, L. Eocene (7).

Monocotyledonopsida

53. *Arecipites barakati* HEGEDŰS, KEDVES and PÁRDUTZ 1972, U. Cretaceous (3).

The examinations to date have led to the following essential conclusions:

The wall of the isosporous Upper Devonian *Archaeotriletes* sp. spore is uniform and composed of anastomosing rodlets of sporopollenin (PETTITT, 1966). Well-definable layers can not be distinguished in the walls of the Lower Eocene *Tmesopsida* and the Upper Cretaceous and Lower Eocene *Filicales* spores. Two parts can be discerned on the basis of the electron affinity,

the external ectexosporium and the internal endexosporium. The superficial ornamentation is exclusively a formation of the ectexosporium.

Similarly, separate layers can not be distinguished in the wall of *Laevigatosporites* cf. *glabratus* (ZERNDT) POTONIÉ and KREMP derived from the heterosporous *Sigillaria* fructificatio; the entire thickness of the exine is composed of the familiar ramifying units of sporopollenin (PETTITT, 1966). The walls of the heterosporous *Pteridophytae* are configurated; PETTITT (1966) distinguished two layers in the wall of *Archaeopteris* cf. jacksoni DAWSON, and named them ectexine and endexine. Very many data are available on the ultrastructures of the spores of the *Hydropterides* species, which are derived primarily from younger, Tertiary deposits. In this connection, mention must be made of the finding of KEMPF (1969a) that the perinaeum of the *Azolla* megaspore resembles that of the *Angiospermae* sporodermis in the arrangement of the foot layer, the columellae and the tectum.

As regards their ultrastructures, the *Pteridospermopsida* sporomorphs can be said to be heterogeneous. For. *Schopfipollenites* sp. it proved possible to detect an ectexine and an endexine, the external with a spongy, and the internal with a lamellar ultrastructure. Layers can not be identified for *Trigonocarpus* sp., while the wall consists of a three-dimensional network of sporopollenin giving a spongy appearance (PETTITT, 1966). Two layers can be discerned in the wall of the *Cystosporites giganteus* (ZERNDT) SCHOPF 1938 megaspore; the external one is composed of fibrils of two different dimensions, while the internal layer is very thin and homogeneous. Finally, the completely homogeneous wall-structure of *Didymosporites* scottic Chaloner is surprising.

Similar to the spore, a two-layered ultrastructure can be identified in the wall of *Florinites* sp. pollen belonging to the *Cordaitopsida.*

The fine structure of the wall of cf. *Araucariacites* v. *Granulatisporites* listed in the *Coniferopsida* is of an angiospermal nature; it is divided into tectum and columellae. The pollen wall of the Jurassic *Spheripollenites* scabratus is markedly of an *Angiospermatophyta* character, and contains tectum, columellae and foot layer. The exine ultrastructure of the *Classopollis* and *Classoidites* genus is extremely complicated, and even more complicated than that of the recent angiosperms. As regards ultrastructure, the two form-genera can be distinguished by the columellae above the endexine.

TEM data for the recent species have been interpreted in that the fossil *Angiospermatophyta* exines consist of ectexine and endexine. The ectexine is built up of three layers (tectum, columellae, foot layer). Thus, the terms ectexine and endexine, which are used in light-microscopic descriptions, do not agree with the ultrastructural results. In general the innermost layer of the ectexine (foot layer) is termed endexine. It must be noted that the detection of the endexine by light-microscopic methods is fairly difficult, because it differs from the ectexine in its ultrastructure or electron affinity. The main morphological types of the pore-wall exine had to be reassessed with the ultrastructural data, e. g. atrium, vestibulum, prevestibulum, colpore. It is a general phenomenon for fossil angiosperm pollen grains too that if there is an endexine below the ectexine, then this is thickened in the pore-wall region. In the *Brevaxones* and *Longaxones* taxons studied the evolutionary value of the endexine is different. In the most primitive *Brevaxones* – *Normapolles* form-genera *(Atlantopollis, Complexiopollis)* the endexine occurs fairly generally, whereas it is

absent from the more developed Lower Eocene types. Thus, the development within this group is revealed by the decrease of the number of layers. On the other hand, it may reappear in the more developed, modern angiosperm pollen grains, but naturally with another taxonomic value. It has so far not proved possible to establish a similar relationship for the *Longaxones* pollen grains, since the occurrence of the endexine is quite general. Three types of endexine occurred within the group; these may possibly also have evolutionary or taxonomic importance later. The ultrastructure of the primitive *Postnormapolles* taxons (e. g. *Subtriporopollenites constans magnus*) can be identified with that of the recent genera, and so the botanical connections can be established even in the cases when there was no possibility for this in the light-microscopic examinations. From an ultrastructural point of view a significant proportion of the Palaeocene – Lower Eocene *Normapolles* are of an *Amentiflorae* type. It was necessary to emend the light-microscopic diagnoses for several fossil *Angiospermatophyta* taxons, while the ultrastructural data too were used in the descriptions of the new taxons.

References

EHRLICH, H. G.—HALL, J. W. (1959): The ultrastructure of Eocene pollen. — Grana Palynologica *2*, 32—35.

HEGEDŰS, M., KEDVES, M.—PÁRDUTZ, Á. (1971): Ultrastructural investigations on fossil Angiosperms exines of Upper Cretaceous. — Advancing Frontiers of Plant Sciences *28*, 317—329.

HEGEDŰS, M., KEDVES, M.—PÁRDUTZ, Á. (1972): Ultrastructural investigations of Upper Cretaceous Angiosperm exines II. — (in press).

KEDVES, M., HEGEDŰS, M.—PÁRDUTZ, Á. (1971): Étude de l'ultrastructure des pollens fossiles des Angiospermes du Crétacé supérieur et du Tertiaire inférieur. — III. Internat. Palynological Conference. Sect. 2 novosibirsk.

KEDVES, M.—PÁRDUTZ, Á. (1970a): Az ultrastruktúra vizsgálatok jelentősége fosszilis Angiospermatophyta pollenszemek fejlődéstörténeti kérdéseinek megoldásában (The importance of ultrastructural studies in the solution of questions of the history of the development of fossil Angiospermatophyta pollen grains) (preliminary communication). — Bot. Közlem. *57*, 57—58.

KEDVES, M.—PÁRDUTZ, Á. (1970b): Études palynologiques des couches du Tertiaire inférieur de la Région Parisienne VI. Ultrastructure de qualques pollens d'Angiospermes de l'Eocène inférieur (Sparnacien). — Pollen et Spores *12*, 553—575.

KEDVES, M.—PÁRDUTZ, Á. (1972a): Alsó eocén kori Angiospermatophyta pollenszemek ultrastruktura vizsgálata (Ultrastructural examination of Lower Eocene Angiospermatophyta pollen grains). — (in press).

KEDVES, M.—PÁRDUTZ, Á. (1972b): Elektronmikroszkópos vizsgálatok fosszilis zárvatermő polleneken (Electron-miscrope examinations of fossil angiosperm pollens). — (in press).

KEDVES, M.—PÁRDUTZ, Á. (1972c): Fosszilis Pteridophyta spórák és Gymnospermatophyta pollenszemek ultrastruktúra vizsgálata (Ultrastructural examination of fossil Pteridophyta spores and Gymnospermatophyta pollen grains). — (in press).

KEMPF, E. K. (1969a): Elektronenmikroskopie der Sporodermis von käinozoischen Megasporen der Wasserfarn-Gattung Azolla. — Paläont. Z. *43*, 95—108.

KEMPF, E. K. (1969b): Elektronmikroskopie der Megasporen von Azolla tegeliensis aus dem Altpleistozän der Niederlande. — Palaeontographica B, *128*, 167—179.

KEMPF, E. K. (1971): Elektronenmikroskopie der Sporodermis von Mega- und Mikrosporen der Pteridophyten-Gattung *Salvinia* aus dem Tertiär und Quartär Deutschlands. — Palaeontographica B, *136*, 47—70.

LEFFINGWELL, H. A., LARSON, D. A.—VALENCIA, M. J. (1970): A study of the fossil pollen Wodehouseia spinata. I. Ultrastructure and comparisons to selected moden taxa. II. Optical microscopic recognition of foot layers in differentially stained fossil pollen and their significance. — Bull. of Canadian Petroleum Geology *18*, 238—262.

PÁRDUTZ, Á., JUHÁSZ, M., DINIZ, F.—KEDVES, M. (1972): Teixeraipollenites globosus n. fgen. et fsp. du Crétacé supérieur de Portugal avec l'étude de l'ultrastructure de leur exine. — (in press).

PETTITT, J. M. (1966): Exine structure in some fossil and recent spores and pollen as revealed by light and electron microscopy. — Bull. B. M. (N. H.) Geol. *13*, 223—257.

PETTITT, J. M.—CHALONER, W. G. (1964): The ultrastructure of the Mesozoic pollen *Classopollis*. — Pollen et Spores *6*, 611—620.

Note. — Only work dealing with the TEM study of fossil sporomorphs is given in the literature references. The manuscript was closed at the end of April 1972.

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ULTRASTRUCTURAL STUDIES ON AMENTIFLORAE POLLEN GRAINS I

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Introduction

In our ultrastructural studies on Lower Eocene *Normapolles* exines it was found that these are derived from primitive *Amentiflorae* taxons (KEDVES and PÁRDUTZ, 1970a,b). In the *Normapolles* taxons the occurrence of the endexine as a function of the geological period is of evolutionary importance (KEDVES, HEGEDŰS and PÁRDUTZ, 1971a, HEGEDŰS, KEDVES and PÁRDUTZ, 1971; 1972). Our ultrastructural examinations have permitted the elucidation of frequently complex pore-wall exines of fossil pollen grains.

In spite of the fact that numerous literature data are known on the ultrastructures of the recent Amentiflorae taxons (AFZELIUS, 1956 – in ERDTMANN, 1956; UENO, 1963a,b; TAKEOKA and STIX, 1963; STONE, REICH and WHITFIELD, 1964, STONE and BROOME, 1971), we considered it necessary to carry out studies in accordance with the following points of view:

1. The description of the exine ultrastructure in those taxons where our knowledge is incomplete.

2. The exact study of the endexine in the species under examination.

3. Interpretation of the concepts of pore, atrium and vestibulum used in the description of fossil taxons in the pore-wall region, on the basis of the ultrastructural data in the recent taxons.

4. Establishment of the ultrastructural nature of special exine formations.

Materials and Methods

The species examined were the following: *Corylus avellana* L., *C. colurna* L., *C. sieboldiana* BLUME, *Cannabis sativa* L., *Carya amara* NUTT., *C. ovata* (MILL.) C. KOCH, *Juglans californica* WATSON, *Myrica gale* L., *Betula alba* L., *B. pubescens* EHRH., *Alnus glutinosa* (L.) GAERTN. The examination material originates from the Botanical Collection of the Natural Science Museum, and we should like to take this opportunity to express our grateful thanks to the Director, JULIA LACZA-SZUJKÓ.

Taking into account the work of STONE and BROOME (1971), our ultra-thin sections were prepared from acetolyzed pollen grains. With more complex light-microscopic morphology serial sections were used in the pore-wall region, as introduced on the Casuarina genus exine, where this was necessary (KEDVES, HEGEDŰS and PÁRDUTZ, 1971b). The results are described genus-wise.

Results

Corylus L. (Fig. 2/1–4)

Interpore-wall exine. – Tectate, perforated with narrow channels, spinae on the surface. The elements of the columellae are varied, mainly ellipsoid and columnar. An endexine can not be distinguished below the foot layer. T/C/F= 3–4/1.5–2/1. (Fig. 2/1)

Pore-wall exine. – The tectum is unchanged in the pore-wall region. The elements of the columellae are accumulated, and small, mainly spherical formations appear below the tectum. The lamellar endexine is marked in the pore-wall region (Fig. 2/2–4). Thus, the annulus is a formation of the columellae and the endexine in this genus. The tectum bends inwards along the pore and combines or almost combines with the foot layer (Fig. 2/4).

Cannabis L. (Fig. 4/1–4).

Interpore-wall exine. – Tectate non-perforate, large spinae on the surface of the tectum. The elements of the columellae are mainly columnar. An endexine can not be discerned below the foot layer. T/C/F=3–4/2/1. (Fig. 4/4).

Pore-wall exine. – The tectum is unchanged in the pore-wall region. The elements of the columellae are strongly increased, mainly by small, spherical elements (Fig. 4/1). The annulus is formed in this way (Fig. 3; 4/2, 3). The foot layer becomes extremely thin at the annulus, and almost breaks, and under it appears endexine with a granular ultrastructure. The tectum bends inwards along the pore, and combines with the tapered foot layer (Fig. 4/2, 3).

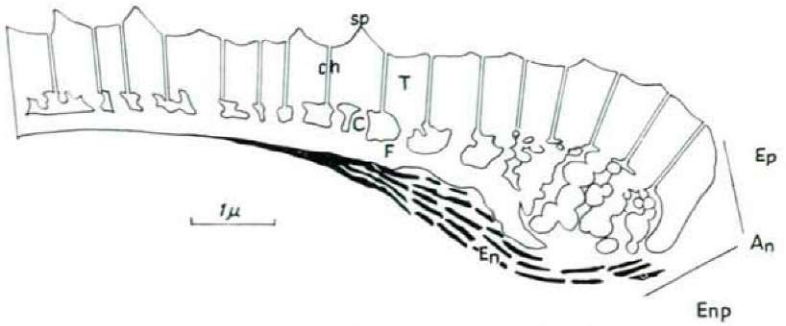

Fig. 1. Ultrastructure of *Corylus colurna* L. exine in the pore-wall region.
T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels, En = endexine, Ep = exopore, Enp = endopore, An = annulus.

Carya NUTT. (Fig. 6/1–4)

Note. – The pore-wall openings are situated sub-equatorially, and the exine becomes thin at the poles. Interpore-wall exine. – Tectate, perforated with narrow channels, the tectum with spinae. The elements of the columellae are spherical or ellipsoid, and frequently anastomize. Along the poles, where

Fig. 2. *Corylus colurna* L.

1. — Ultrastructure of the interpore-wall exine. M : 25,000x.
2—4. — Ultrastructure of the pore-wall exine in serial section. M : 25,000x.
T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels, En = endexine, An = annulus, P = pore.

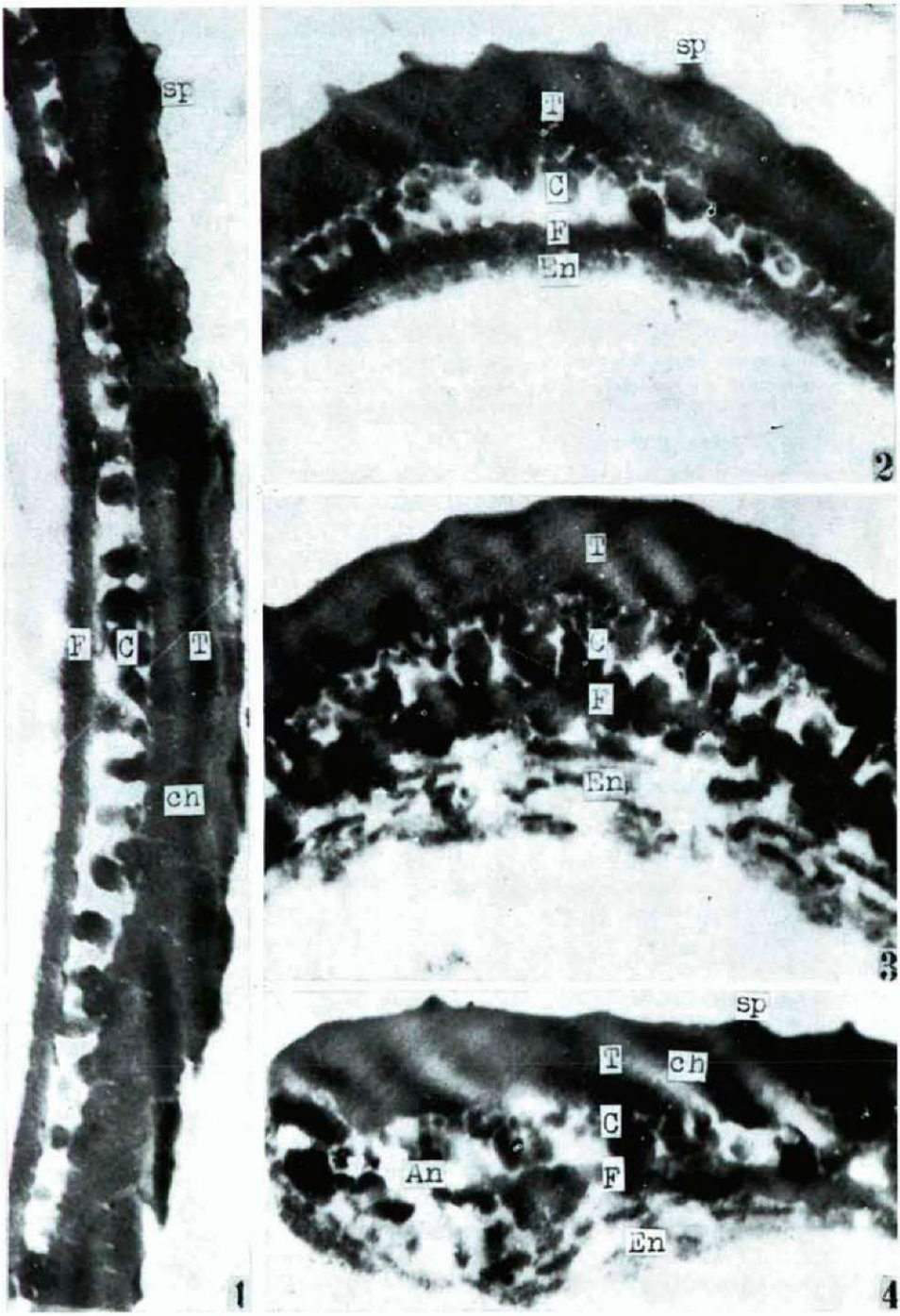

Fig. 2

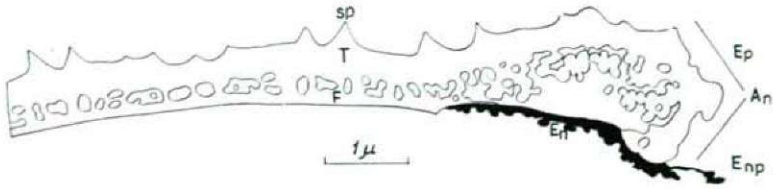

Fig. 3. Ultrastructure of *Cannabis sativa* L. exine in the pore-wall region.
T = tectum, C = columellae, F = foot layer, sp = spinae, En = endexine, Ep = exopore, Enp = endopore, An = annulus.

Fig. 4. *Cannabis sativa* L.

1. — Ultrastructure of the pore-wall exine in the vicinity of the pore-wall region. M : 25,000x.
2. — Ultrastructure of the pore-wall exine in the pore-wall region. M : 50,000x.
3. — Ultrastructure of the pore-wall region in the pore-wall region. M : 25,000x.
4. — Ultrastructure of the interpore-wall exine. M : 25,000x.

T = tectum, C = columellae, F = foot layer, sp = spinae, En = endexine, An = annulus, P = pore.

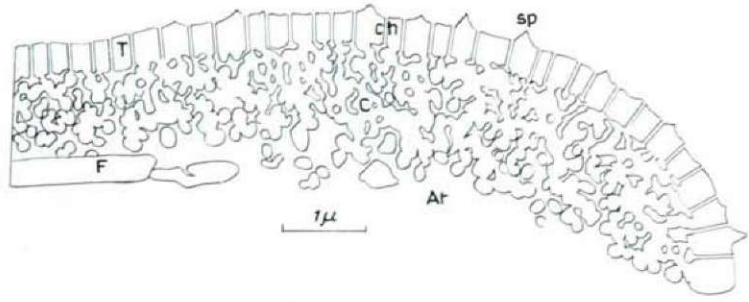

Fig. 5. Ultrastructure of *Carya amara* NUTT. exine in the pore-wall region.
T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels, At = atrium.

the exine becomes thin, the tectum and the foot layer are unchanged, and the elements of the columellae are generally in two rows – T/C/F=1.5/1/1 – but in the vicinity of the equator they are generally observed in eight rows – T/C/F=1.5/4/1 (Fig. 6/1, 4).

Pore-wall exine. – The tectum bends inwards along the pores, its thickness unchanged, and the columellae too become narrow only along the pores. There is no annulus, the foot layer is broken in the pore-wall region, and hence there is in essence an atrium (Fig. 5; 6/2, 3).

Juglans L. (Fig. 8/1–3)

Interpore-wall exine. – Tectate, perforated with narrow channels, relatively large spinae on the tectum. The elements of the columellae are spherical or ellipsoid, and are arranged in four rows on the tapering polar part of the exine, and generally in six rows in the vicinity of the equator. T/C/F=1.5–2/1.5/1 or 1.5–2/2–2.5/1. There is no endexine below the foot layer.

Pore-wall exine. – The tectum agrees with that of the Carya genus in the pore-wall region. The elements of the columellae are accumulated, however, in 10–14 rows, and form an annulus. The foot layer is broken in the pore-wall region, and an atrium is formed (Fig. 7; 8/1).

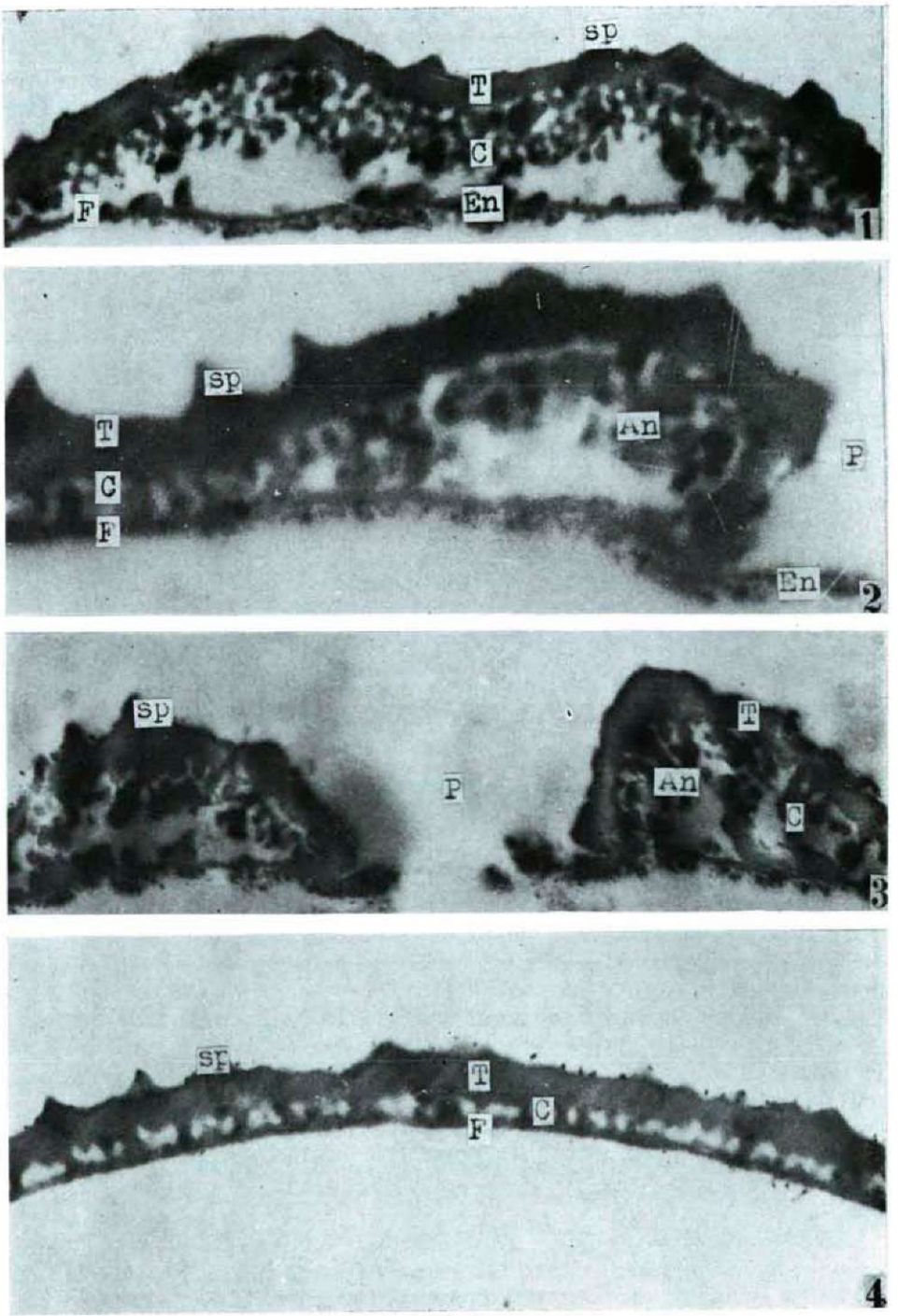

Fig. 4

Fig. 6. 1. — Ultrastructure of *Carya amara* NUTT. interpore-wall exine with the tapering columellae at the poles. M : 25,000x.
2. — Ultrastructure of *Carya alba* NUTT. pore-wall exine. M : 10,000x.
3. — Ultrastructure of *Carya amara* NUTT. exine in the pore-wall region. M : 10,000x.
4. — Ultrastructure of *Carya amara* NUTT. interpore-wall exine in the equatorial region. M : 50,000x.
T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels, At = atrium.

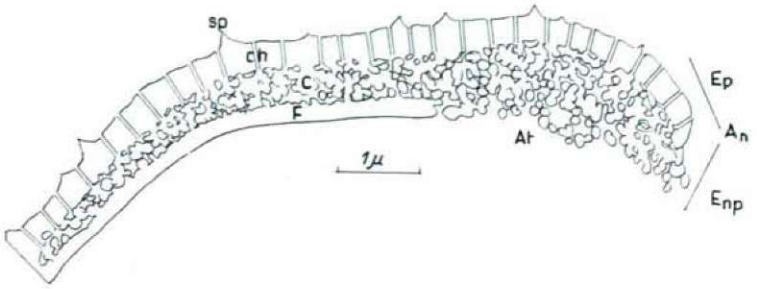

Fig. 7. Ultrastructure of *Juglans californica* WATSON exine in the pore-wall region.
T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels, At = atrium, An = annulus, Ep = exopore, Enp = endopore.

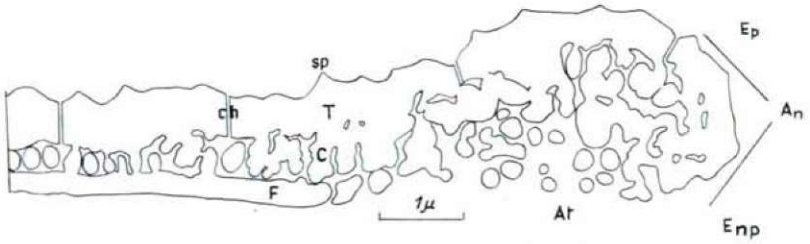

Fig. 9. Ultrastructure of *Myrica gale* L. [= *Gale palustris* (LAM.) CHEVAL.] exine in the pore-wall region.
T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels, At = atrium, An = annulus, Ep = exopore, Enp = endopore.

Myrica L. (Fig. 10/1–5)

Interpore-wall exine. – Tectate, rarely perforated with narrow channels, spinae also rarely situated on the surface. The elements of the columellae are spherical, ellipsoid, or rarely columnar, and arranged in one, or rarely two rows. There is no endexine below the foot layer (Fig. 10/5). T/C/F = 2–3/–1.5/1.

Pore-wall exine. – The thickness of the tectum is unchanged in the pore-wall region, and it bends inwards along the exogerminalia. The elements of the columellae form an annulus by their accumulation, and here the structural elements frequently anastomize. The foot layer breaks in the vicinity of the pore-wall region, partially divides up, and in this way the atrium is formed (Fig. 9; 10/1–4).

Betula L. (Fig. 12/1, 3, 4)

Interpore-wall exine. – Tectate, perforated with narrow channels, decorated with spinae on the surface (Fig. 12/1). The columellae and the foot layer

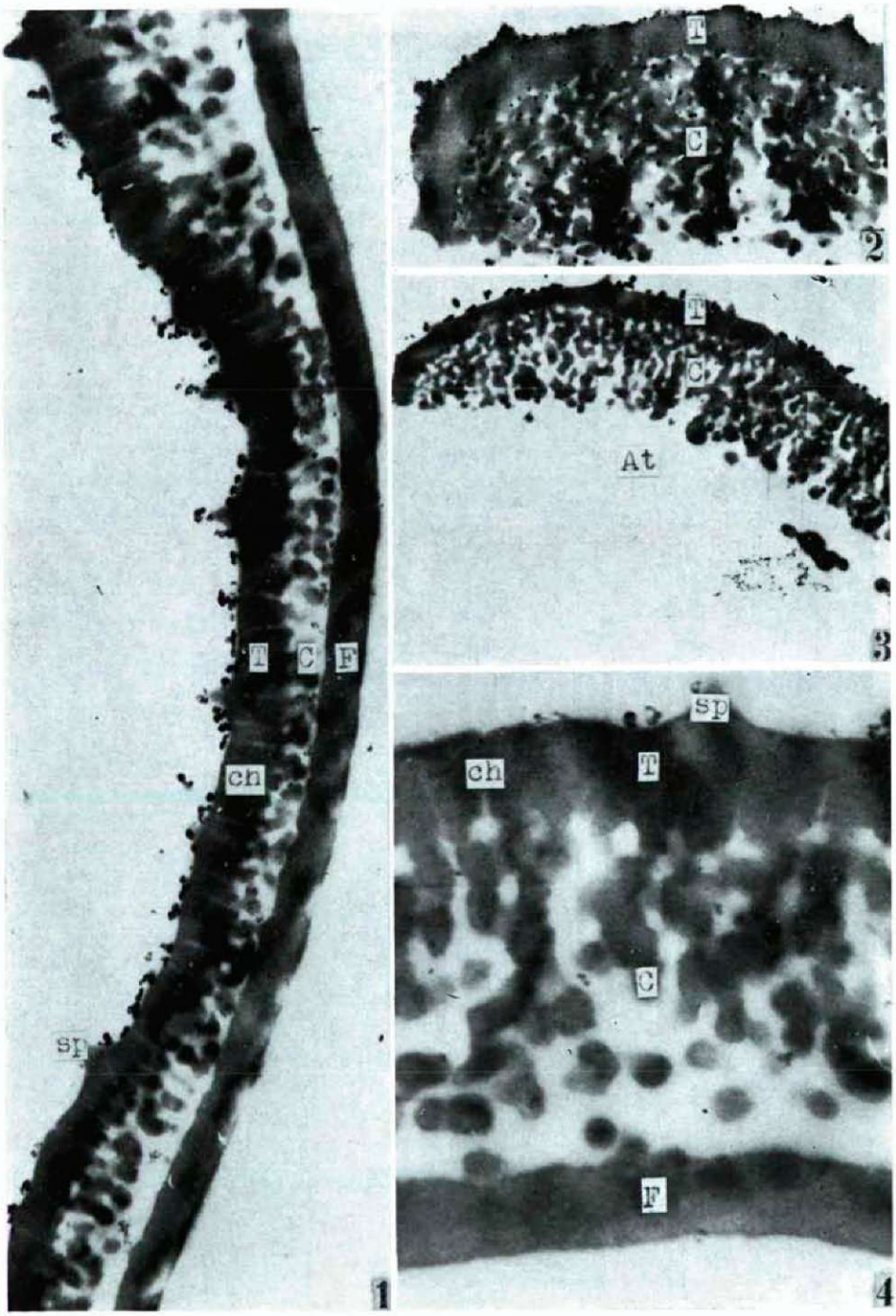

Fig. 6

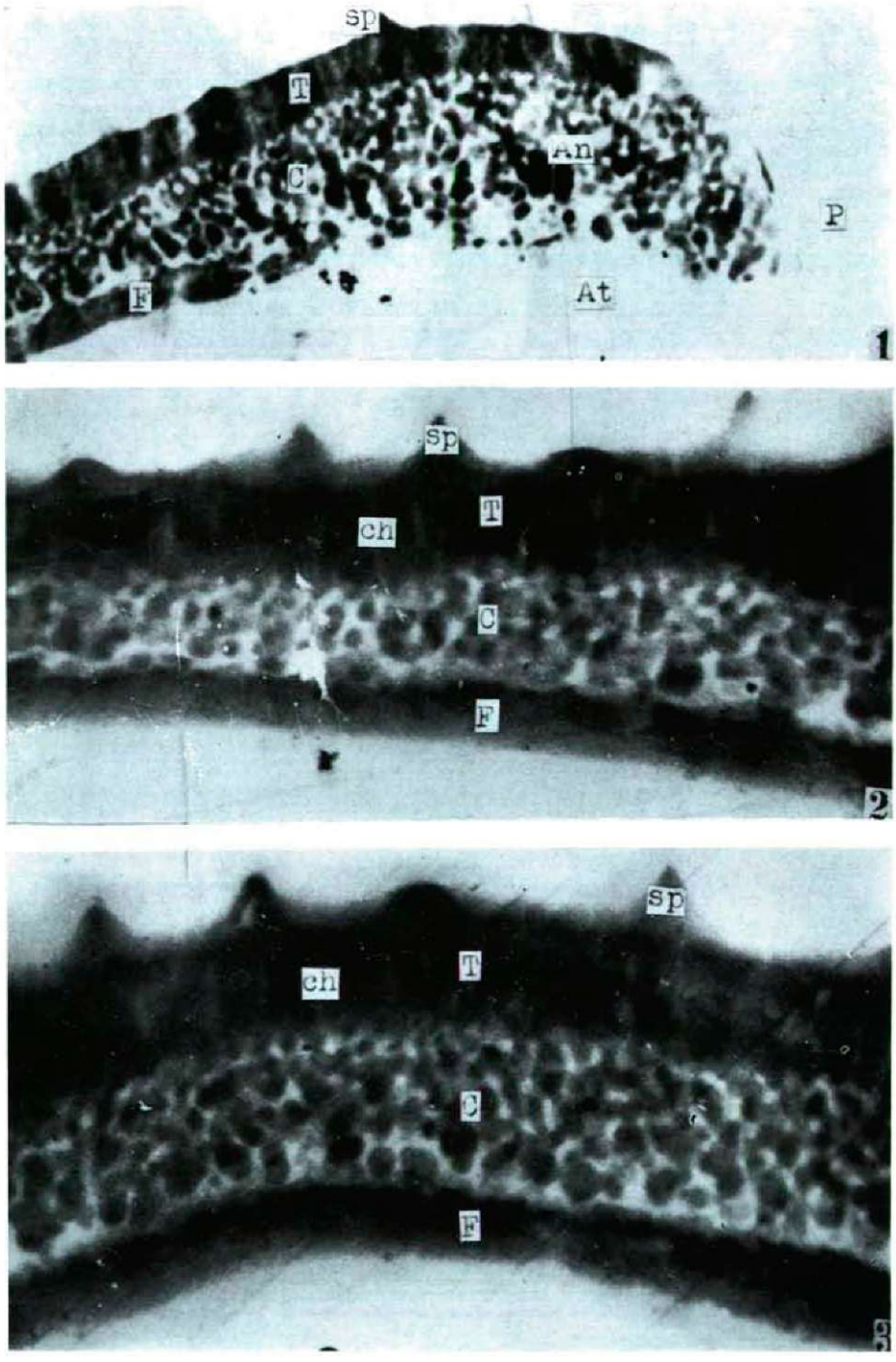

Fig. 8

Fig. 8. *Juglans californica* WATSON

1. — Ultrastructure of the pore-wall region. M : 25,000x.
2. — Ultrastructure of the interpore-wall region in the vicinity of the pole, with the tapering columellae. M : 50,000x.
3. — Ultrastructure of the interpore-wall exine in the equatorial region. M : 50,000x.

T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels, At = atrium, P = pore.

are very narrow, in contrast with the tectum. T/C/F=6–7/2/1. The columellae are single-rowed, and their elements have various shapes, ellipsoid, columnar, etc.

Fig. 10. *Myrica gale* L. [= *Gale palustris* (LAM.) CHEVAL.]

1,2,4. — Ultrastructure of the pore-wall exine in serial section. M : 10,000x.
3. — The columellae in the pore-wall region. M : 25,000x.
5. — Ultrastructure of the interpore-wall exine. M : 50,000x.

T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels, At = atrium, An = annulus.

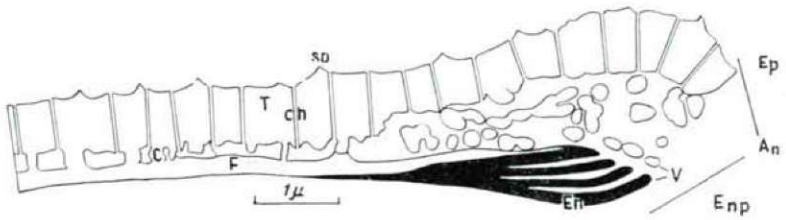

Fig. 11. Ultrastructure of *Betula alba* L. exine in the pore-wall region.
T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels, En = endexine, V = vestibulum, An = annulus, Ep = exopore, Enp = endopore.

Pore-wall exine. – The tectum is unchanged in the pore-wall region, while the elements of the columellae are in part larger than in the interpore wall, and in part accumulate and form an annulus. The foot layer breaks before the pores, and separates a little from the columellae (Fig. 11; 12/3, 4). There is an endexine of marked lamellar ultrastructure below the foot layer in the pore-wall region.

Alnus MILL. (Fig. 12/2, 5)

Interpore wall exine. – Tectate, perforated by narrow channels, with wide – – based spinae on the surface. The elements of the columellae are two- and three-rowed, spherical, ellipsoid, or irregular in shape (Fig. 12/2). T/C/F= 2–3/1–1.5/1.

Pore-wall exine. – The tectum becomes narrower in the direction of the exogerminalia. The elements of the columellae are strongly accumulated, in 10–12 rows, and also somewhat larger than in the interpore-wall region. The foot layer too is thicker before the pore-wall openings, and under it is a narrow endexine of lamellar ultrastructure. The foot layer and the endexine are a little separated from the columellae (Fig. 12/5; 13).

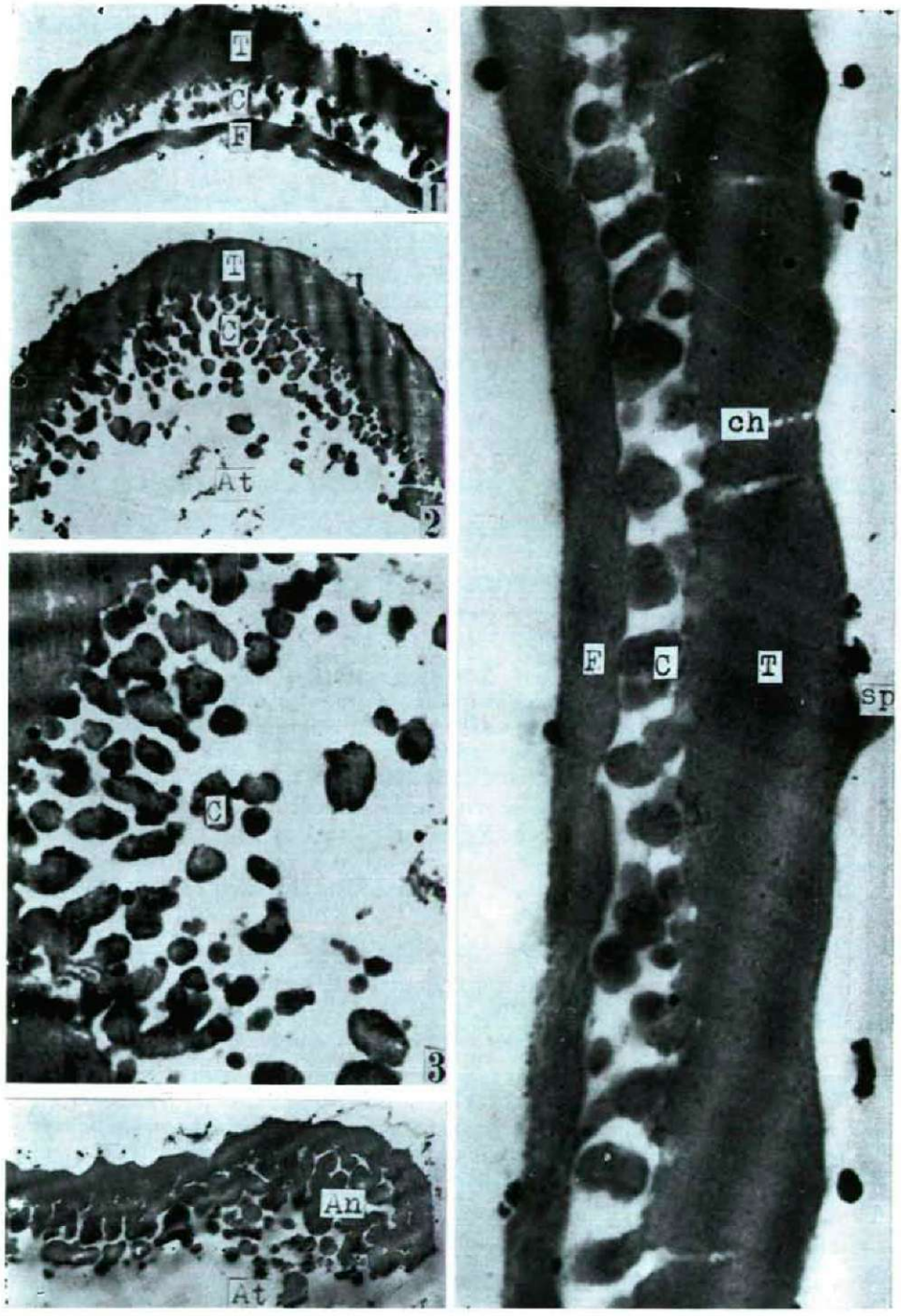

Fig. 10

Discussion of the results

From a comparison of our results with the findings of STONE and BROOME (1971), the following conclusions can be arrived at:

The occurrence of the channels is general in the tectum in the *Betulaceae, Corylaceae, Juglandaceae* and *Rhoipteleaceae* families, they are rare in the *Myricaceae* species studied, and they are not found in *Cannabis sativa;* thus, to a certain extent they are of taxonomic importance. The spinae on the surface are a general feature with Amentiflorae; they are probably of ecological significance, and a result of wind-pollination. According to TAKEOKA and STIX (1963), its finer morphology is of taxonomic value, but this can be evaluated primarily with a scanning electron-microscope method. On the basis of the species examined, the finer morphology of the columellae for taxonomic distinction, primarily of the genera.

According to our present knowledge, the endexine does not occur in the *Juglandaceae* genera *(Carya, Engelhardtia, Juglans)* and in the *Myricaceae.* In the Corylaceae and Betulaceae genera there is an endexine with lamellar ultrastructure in the pore-wall region, while in *Cannabis sativa* there is an endexine with granulated ultrastructure. As regards the ultrastructure, this latter species is very well distinguished not only by its characteristic endexine, but also by the fact that there are no channels in the tectum.

The annulus is generally formed by means of the accumulation of the elements of the columellae, and in the *Corylaceae* and the *Betulaceae* the endexine too participates in its formation. In *Cannabis sativa* the endexine is not important from this respect.

Fig. 12. 1. — Ultrastructure of *Betula alba* L. interpore-wall exine. M : 25,000x.
2. — Ultrastructure of *Alnus glutinosa* (L.) GAERTN. interpore-wall exine. M : 25,000x.
3,4. — Ultrastructure of *Betula alba* L. pore-wall exine. M : 10 000x.
5. — Ultrastructure of *Alnus glutinosa* (L.) GAERTN. pore-wall exine. M : 10,000x. T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels, En = endexine, An = annulus.

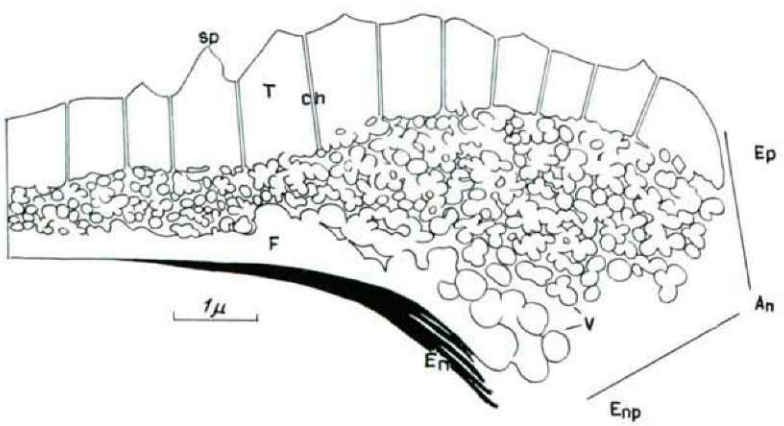

Fig. 13. Ultrastructure of *Alnus glutinosa* (L.) GAERTN. exine in the pore-wall region. T = tectum, C = columellae, F = foot layer, sp = spinae, ch = channels. En = endexine, V = vestibulum, An = annulus, Ep = exopore, Enp = endopore.

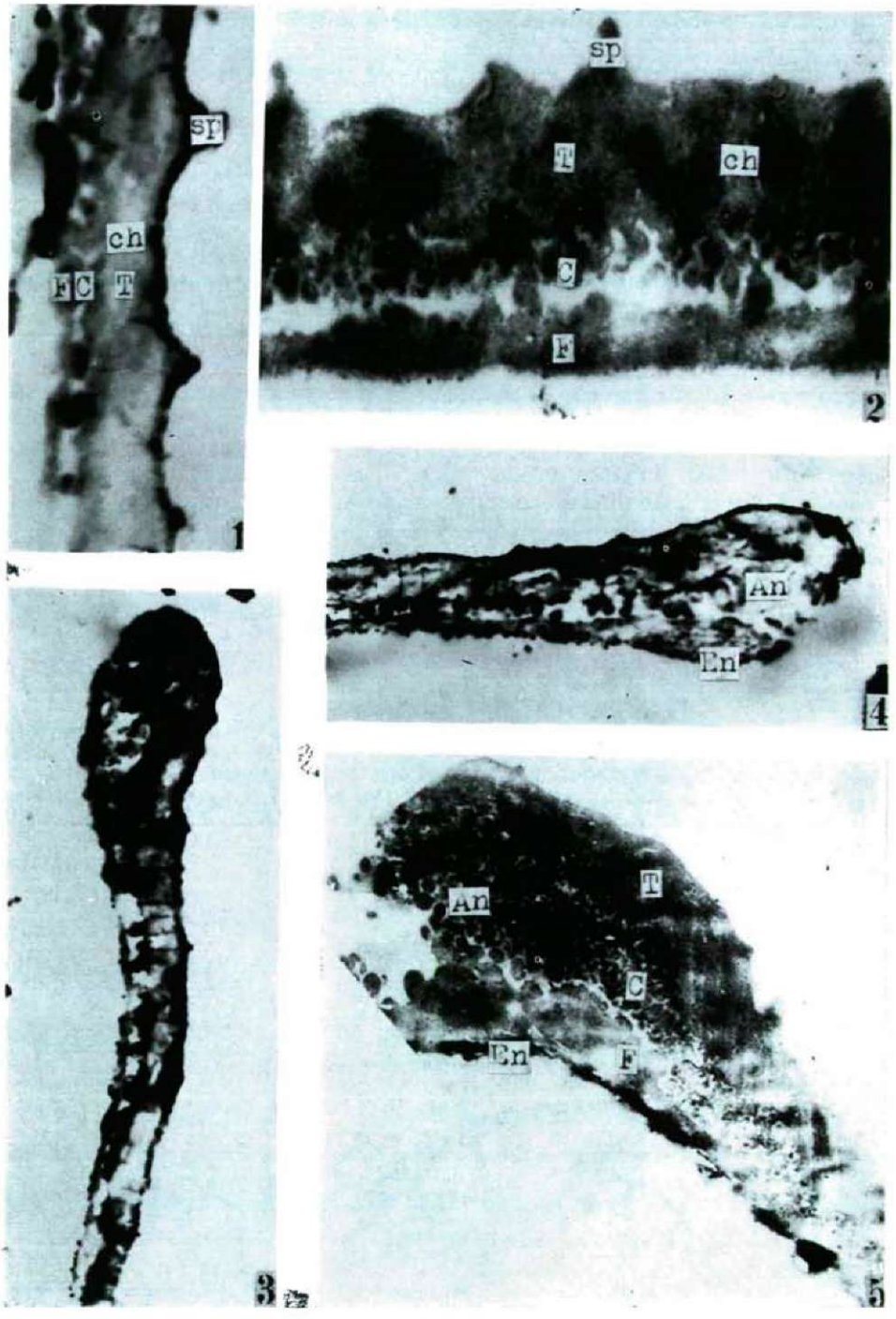

Fig. 12

In the pore the tectum and the foot layer combine *(Cannabis sativa),* or nearly combine: the two layer bend together (e. g. the *Corylus* genus). In the atrium the foot layer breaks before the pore-wall region; this is typical for the *Myrica* and the *Engelhardtia* genera, but essentially the same is found for the *Carya* and *Juglans* species too. In the case of the vestibulum the foot layer (together with the endexine) separates from the columellae in the pore-wall region and bends inwards towards the centre of the pollen. This is characteristic for the *Betula* and the *Alnus* genera. The „fainter zones" at the poles for the pollen grains of the *Carya* and *Juglans* genera are formed by the tapering of the columellae (cf. STONE, REICH and WHITFIELD, 1964).

Summary

1. In the Amentiflorae taxons the exine ultrastructural features are partially of ecologic value (e. g. the spinae on the tectum), and partially of taxonomic value.

2. From a taxonomic point of view the channels, the finer structure of the columellae, the endexine and the pore-wall region formations (pore, atrium, vestibulum) or the tapering zones sometimes occurring on the poles of the pollen grains can be used.

3. The features identified in the recent taxons are identical to the ultrastructural characteristics established on the fossil pollen grains (e. g. the atrium in *Plicapollis pseudoexcelsus).*

References

ERDTMAN, G. (1956): Current trends in palynological research work. — Grana Palynologica *1,* 128—139.

HEGEDŰS, M., KEDVES, M.—PÁRDUTZ, Á. (1971): Ultrastructural investigations on fossil Angiosperms exine of Upper Cretaceous. — Advancing Frontiers of Plant Sciences *28,* 317—329.

HEGEDŰS, M., KEDVES, M.—PÁRDUTZ, Á. (1972): Ultrastructural investigations of Upper Cretaceous Angiosperm exines II. — (in press).

KEDVES, M., HEGEDŰS, M.—PÁRDUTZ, Á. (1971a): Étude de l'ultrastructure des pollens fossiles des Angiospermes du Crétacé supérieur et du Tertiaire inférieur. — III. Internat. Palynological Conference. Sect. 2, Novosibirsk.

KEDVES, M., HEGEDŰS, M.—PÁRDUTZ, Á. (1971b): Ultrastructure investigations on the exine of the genus *Casuarina* L. (Short communication). — Acta Biol. Szeged *17,* 63—65.

KEDVES, M.—PÁRDUTZ, Á. (1970a): Az ultrastruktúra vizsgálatok jelentősége fosszilis Angiospermatophyta pollenszemek fejlődéstörténeti kérdéseinek megoldásában (The importance of ultrastructural studies in the solution of questions of the development of fossil Angiospermatophyta pollen grains (preliminary communication). — Bot. Közlem. *57,* 57—58.

KEDVES, M.—PÁRDUTZ, Á. (1970b): Études palynologiques des couches du Tertiaire inférieur de la Région Parisienne VI. Ultrastructure de quelques pollens d'Angiospermes de l'Eocène inférieur (Sparnacien). — Pollen et Spores *12,* 553—575.

KUPRIANOVA, L. A. (1965): The palynology of the Amentiferae (in Russian). — Moscow—Leningrad.

STONE, D. E., REICH, J.—WHITFIELD, S. (1964): Fine structure of the walls of *Juglans* and *Carya* pollen. — Pollen et Spores *6,* 379—392.

STONE, D. E.—BROOME, C. R. (1971): Pollen ultrastructure: Evidence for relationship of the *Juglandaceae* and the Rhoipteleaceae. — Pollen et Spores *13*, 5/14.

TAKEOKA, M.—STIX, E. (1963): On the fine structure of the pollen walls in some Scandinavian *Betulaceae*. — Grana Palynologica *4*, 161—188.

UENO, J. (1963a): On the fine structure of the pollen walls of Angiospermae. III. *Casuarina*. — Grana Palynologica *4*, 189—194.

UENO, J. (1963b): The stratigraphical structure of the pollen walls of Dicotyledoneae. I. Ranales and Amentiferae. — Acta Phytotax. Geobot. *19*, 137—141. (Japanese, with English summary.)

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EFFECTS OF FOIL COVERS OF DIFFERENT COLOURS ON THE TISSUE STRUCTURE OF THE LEAVES OF LETTUCE, SPINACH, AND GARDEN SORREL

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Introduction

Of late years, plastic foils have been used increasingly for growing garden plants. In Hungary, the surface of foil beds was 1 million sq. m. in 1965, and their use has considerably grown from year to year (SOMOS, 1967). With foil covering the vegetation time can be prolonged considerably both in spring and autumn months, in this way providing an opportunity of producing early and late primeurs under field conditions.

Somos and co-workers have dealt with the theoretical and practical problems of foil covering for some decades. They have elaborated several „foil cent" types and agricultural techniques for growing certain garden plants.

For covering they have used colourless PVC and polyethylene foils. Since the 1950s, more and more researchers have atempted to apply coloured plastic foils under experimenthal conditions. In Hungary, these investigations were begun by HORVÁTH (1965) in 1962. The theoretical basis is the old recognition (SENEBIER, 1785; later TIMIRYAZEV, 1875) that lights of different colours exert different effects on plants.

Literature survey

A change in the strength and quality of the light is very important in the case of plants, as by means we can exert an influence on the photosynthesis, the fundamental plant function. As a result, there is a change in the amount and composition of the organic matter in the plant, and, as shown by the results of several researches, the metabolic changes taking place in this way bring about external morphological, phenomenological, anatomical, etc. alterations.

By prolonging the time of illumination, we generally increase the production of organic matter and, parallel with this, the growth of the plant as well. Illumination providing intense photosynthesis usually also makes good growth possible.

According to the findings of PÁL (1966) and HORVÁTH and KOLTAY (1967), the plant height is influenced rather by the strength of illumination than by the spectral range. The stem length and leaf size are increased by a decreased energy level. The optimum strengths of illumination are very different

for the individual plant species: in most cases, full natural sunshine is not necessary.

In the development of the intensity of photosynthesis the leaf thickness too plays an essential part. If the leaves are very thick, the more absorptive light is absorbed already in the surface cells. For these leaves, the effect maximum is shifted towards the orange. Apart from the leaf thickness, the intensity of photosynthesis is considerably influenced by the structure of the assimilating parenchyma, the number of leaf vessels and the quantity of supporting tissues (McCLEDON, 1962).

For the cabbaging (heading) of lettuce, a balance of illumination and temperature is essential. DULLFORCE (1969) investigated under conditioned circumstances the light and temperature values that are critical for cabbaging. At a permanent temperature of 13 °C they could achieve cabbaging if the visible energy was 10 cal/sq. cm/day. The leaf surface of the seedlings developing at 21 °C being larger, these plants make better use of the decreased winter light amount.

The morphological effects of lights of different qualities and intensities are strongly interdependent. The interdependence may be so strong that the effect of a ligth of a given quality becomes the opposite at a different energy level. Red light, for example, increases the longitudinal growth of leaves at a low energy level but decreases it at a high energy level (NUERNBERGK, 1966). According to VINCE (1956), at the same energy level the size of leaf blades grows at a rate depending upon the spectral band, but there are important differences between individual plants. With regard to the leaf surface, HORVÁTH (1965) emphasizes the marked influence of the spectral composition but this differs according to the plant species. For pea, an increase in the proportion of the blue spectral range enralges, whereas that of the red spectralband reduces the leaf surface, while for paprika the blue reduces and the yellow enlarges it.

According to FORTANIER (1954), the number of leaves is not affected by the quality of the light. On the other hand, HORVÁTH (1965), investigating the effects of different spectral ranges, obtained a significant difference: for paprika, considerably more leaves developed in the phytotrone under yellow fluorescence than under F_{33} „orange" lamps.

The effect exterted by the spectral composition of the light on the stem lengthening changes according to the plants, too depending upon the intensity of illumination. In connection with the lengthening of the internodes too, as for the size of the leaf blades VINCE (1956), generalizes the finding that at an identical energy level the length of the internodes increases depending on the wavelength.

HORVÁTH (1965), investigating the dry matter production of plants under colourless and yellow foils and of control plants, achieved higher values than for plants grown under red and blue foils. In his opinion, with the exception of highly light-demanding plants the organic matter production can be increased by decreasing the level of light energy (applying plastic foils) and ensuring the suitable spectral range. Concerning the organic matter production, he regards the quality of the light as more important than its intensity. NUERNBERGK (1966) relates the effect of the spectral composition on the organic

matter production with the tissue structure of the leaf. Intensification of the strongly absorbing spectral ranges, i. e. the blue and red increases the productivity of photosynthesis only in the case of thin leaves.

The quality of the light influences the plant tissues, too in a differentiated way. LÁSZLÓ–SZALKAI (1964), HORVÁTH (1965) and SIMON–SZEGEDI (1969, 1970) studied and evaluated the morphological, anatomical and productivity changes of some domesticated plants, under lights of different intensities and spectral ranges. The change could be evaluated above all in the supporting tissue system and in the size-fluctuation of the ligneous part of the conductive tissue; the least change was in the phloem and cambium. The increase in the proportion of the ligneous part and the supporting tissue system takes place to the detriment of the medullary parenchyma. The proportion of the ligneous part and the supporting tissue system is the highest in a light of spectral composition shifted towards the blue. Therefore, a relation of positive direction is achieved between both tissue systems as well as the increase in the ratio of the carbohydrate compounds (HORVÁTH, 1965).

SIMON and SZEGEDI (1969, 1970), analysing the stem tissue structure of pea grown under foil tents of different colours, established that the largest phloem developed in the plants in uncovered plots, and the smallest in those covered with red foil. The uncovered, colourless and yellow variations accumulated almost twice as much ligneous element as developed under green, blue and red foils.

The results of several investigations show that the effects of varying the spectral composition of the light by means of plastic foils are not the same for different species. It is important therefore, to investigate these changes for the most important garden plants.

Lactuca, *Spinacia* and *Rumex* species were first investigated, with regard to the effects of light of different intensities and spectral compositions on the external morphology and tissue structure of the leaf.

Materials and Methods

The investigations took place in 1970, under field conditions, in the Botanical Gardens of the Attila József University, Szeged. The following economically important primeur plants were investigated:

Lactuca sativa L. var. *cap.* convar. Cabbage-lettuce „King of May", „Budai" and „Soroksári".

Spinacia oleracea L. var. *glabra* convar. Spinach „Eskimo", „Matador", „Viroflay".

Rumex acetosa L. var *hortensis* convar. „Largeleaved" Sorrel.

The seeds were sown on May 14th (May—June experiment) and on June 25th (June—July experiment). Spinach was grown for five weeks, cabbage-lettuce for six weeks, and sorrel for seven weeks. (Sorrel was investigated only during the May—June growing.)

The plants were grown under field conditions, in plots of 4 sqm. There were five variants, each duplicated. Lights of different spectral compositions were provided by means of coloured foil covers. The foils were perforated in 25 per cent of the total surface.

The experimental area was an irrigated meadow soil. The covering with a perforated foil (HORVÁTH, 1965) changed the air temperature to only a comparatively low extent (Table 1)

The total radiation is decreased by the different means of covering to different degrees and the permeability is different in the individual spectral ranges. In the case of coloured foils (HORVÁTH, 1965), the shifting of the spectral composition of the towards the foil colour can be well proved in every case (Table 2).

Table 1

variant	total radiation	air temperature
uncovered control	100%	29,2 °C
colourless foil	75%	29,4 °C
blue foil	38%	29,0 °C
yellow foil	62%	28,6 °C
red foil	37%	27,6 °C

Table 2

variant	energy distribution as a percentage of the energy in the spectral range 400—700 nm.					
	1	2	3	4	5	6
uncovered control	14	25	25	7	10	19
blue foil	22	32	22	5	5	14
colourless foil	14	24	26	8	12	19
yellow foil	10	22	26	9	12	21
red foil	11	14	11	4	14	46

1 = violet (400—436 nm)
2 = blue (436—495 nm)
3 = green (495—566 nm)
4 = yellow (566—589 nm)
5 = orange (589—627 nm)
6 = red (627—700 nm)

Table 3

Month	mean temperature °C			precipitation mm			number of sunny hours		
	1970	mean	deviation	1970	mean	deviation	1970	mean	deviation
May	14,9	16,8	— 1,9	35	61	—26	184	258	—74
June	20,9	20,0	+ 0,9	61,2	63	—6,8	261	271	—10
July	21,8	22,3	— 0,5	50,1	51	—0,9	284	309	—25

Mean = 50-year average

The main meteorological data during cultivation are given in Table 3, compared with the 50-year mean (placed at our disposal by the Department of Climatology, Attila József University, Szeged).

In the course of the outside morphological and phenological investigations the following characteristic were taken into consideration:

1. number of leaves
2. height of the plant
3. maximum width and length of the leaf-blades
4. fresh weight of leaves
5. appearence of flowers and shooting of the seed-stalks

The maximum length of the leaf-blade (lamina) was measured along the main vessel, and its at the widest part of the leaf. The values obtained in this way are approximately suitable for characterizing the size of the lamina. On collection of the material for investigation, we recorded the fresh weight of the vegetative organs. From under foils of identical colours, we weighed 20 individuals, each of average development. The number of leaves and the size of leaf-blades were investigated on five occasions (2, 3, 4, 5, 6, — weeks old).

For histological processing five to ten well-developed leaves were collected from nods 3—4—5 for every treatment. The middle third of the leaves was used for dissections. The material collected was fixed until processing in a mixture of absolute alcohol, formalin and distilled water in a ratio of 3 : 1 : 1, or stored in 40% ethanol. The leaves were prepared by maceration, and embedded in celloidin. The section were made with a microtome; after being cleaned they were stained progressively with Ehrlich's iron haematoxylin and fixed in Canada balsam.

On the adaxial and abaxial surfaces of the leaves and the leaf cross-section the following recordings/50 fields of sight each were carried out:

1. stoma number per sq. mm,
2. thickness of leaf-blade,
3. thickness of adaxial epidermis and palisade parenchyma
4. thickness of abaxial epidermis and spongious parenchyma
5. proportion of phloem and cambium in the main vessels
6. proportion of the ligneous part in the main vessels.

Projected on transparent paper, the tissue parts of the conductive bundles in the main vessel were drawn round. The proportions of the ligneous and phloem tissues were estimated on the basis of the weight of the transparent paper, corresponding to the surface size of the tissue regions.

The properties recorded show a curve of normal distribution. The reliability of the data was evaluated, therefore, by variance analysis, F and T tests. The calculations were carried out with an M : 3 computer in the Cybernetic Laboratory, Attila József University, Szeged.

Discussion and evaluation of results

The changes taking place as a result of the foil covers of different colours have been evaluated with regard to the intensity of illumination, and the spectral composition of the light. In the evaluation of the results, we have taken into consideration the favourable properties of the edible primeurs.

1. Number of leaves

In the May–June growing, the most leaves were produced by the plants under yellow and colourless foils, except for sorrel. The fewest leaves found under the blue and red foils.

For garden sorrel, covering with foil is not favourable as most leaves develop in full sunshine. For this plant there is a linear relation between the intensity of illumination and the number of leaves (Table 4).

Table 4. May—June experiment

variant	*L. sativa* var. *cap.* convar.			*S. oleracea* var. *glabra* convar.			*R. acetosa* var. *hort.* convar.
	„King of May"	Buda	Sorok-sár	Eskimo	Matador	Viro-flay	Large-leaved
uncovered control	20	18	15	10	9	20	15
colourless	22	23	22	12	12	12	12
yellow	18	22	20	13	15	15	11
blue	8	12	8	10	12	25	7
red	10	15	12	8	10	12	10

Table 5. June—July experiment

variant	*L. sativa* var. *cap.* convar.			*S. oleracea* var. *glabra* convar.		
	„King of May"	Buda	Sorok-sár	Eskimo	Matador	Viro-flay
uncovered control	15	14	16	14	11	11
colourless	16	11	13	16	15	9
yellow	11	11	10	10	10	14
blue	10	11	9	16	9	13
red	15	12	15	12	15	12

For spinach, we have observed a reaction differing according to the species. In the case of Eskimo and Matador, similarly to the three lettuce species, the number of leaves is increased by the yellow and colourless foils. In the case of Viroflay, however, the leaf number is increased by the blue foil.

In the period June–July, for cabbage lettuce the most leaves are produced for the control and under the colourless foil but this value is also approached with red the foil covering. Spinach gave more leaves as a result of covering, but this effect was exerted with foils of different colours, depending on the species, (blue and yellow for Viroflay, colourless and red covers for Matador and Eskimo.)

The number of leaves developing under different foil covers are listed in Tables 4 and 5.

2. Height of plants

Under the foils, increase in the intensity of illumination leads to a longer stem; under a colourless foil it is even longer than that of the uncovered control. The degree of lengthening differs according to the species. For the lettuce „King of May", the lengthening of the internodes is not extensive, with the important result of the „lettuce heads" thus being more compact. A major lengthening of the stem is associated with smaller leaves, which in the case of lettuces, is disadvantageous from a practical point of view.

In the June–July experiment, the stem was the longest under red foil; as compared with the firts experiment, however, the plants were lower.

In the early period the blue and red foils, i. e. low light intensity, are favourable to lettuce from the point of view of heading.

3. Size of leaf-blades

It can be established from the results of the first experiment that the leaf size grows in linear proportion to the intensity of illumination, but the degree of growth differs for the individual species. The leaf surface of the lettuce species is enlarged the most by an increase in the intensity of illumination. For sorrel, during the foil covering, a contrary tendency may be observed: the leaf size is increased by a decrease in the intensity of illumination.

In the June–July growing, of the coloured foils the red ones show a marked spectral effect. The lettuce increases its leaf surface to about twice that of control. This spectral effect is manifested in the case of spinach during both cultivations, but in the first experiment the activity of the meristems is increased to a higher degree by the red spectral range.

4. Fresh weight

We have recorded the fresh weight of the cabbaging lower leaves, as from a practical point of view this is more important than the dry weight. In the first experiment, there is a close, positive correlation between the intensity of illumination and the fresh weight. From among the three sorts of cabbage lettuces, the fresh weight of the leaves of „King of May" was increased the most by the higher energy level. At a low light intensity, the loss of weight as a result of increasing the blue spectral range is very considerable. In the second experimental series, the effect of the energy level does not exert any essential change. Here the spectral effect comes to the front; the weight increases progressively in the direction of the enhanced long-wave red rays. These results can be correlated with the number of leaves and their size, where the degree and direction of the changes are similar to these. The difference between the two experimental periods is great; in May–June, owing to the increasing light intensity, the leaf production generally also increases, but July it decreases. The contradiction that, as a result of being overshadowed by the foils, in June–July at the lower energy level there is more fresh weight than with the full light of the sun, can be explained in that the high energy level of the natural light is not optimum for these plants if not coupled with appropriate temperature and water supply. This was proved by Mattei (1967), who investigated the effect of shade on cabbage lettuce. The highest leaf production was given by a 50% decrease of the light intensity.

„King of May" is worth mentioning because the proportion of the marketed cabbaging stem-leaves in the total weight (stem + leaf) is the highest. In the other two species, the leaf production is lower because of the increased stem elongation.

5. Phenological properties

The species investigated also have some different properties from a phenological point of view. As regards the cabbage lettuces sown in May, at the time of collecting (sixth week), the reproductive organs of „King of May" had not developed fully in any of the variants. For „Buda" the flower-buds appeared during every treatment. In the case of „Soroksár", under blue and red foils, the reproductive organs developed five to seven days later. We have followed the shooting of the seed-stalks of spinach, too. The favourable effect of the red foil is manifested in the retardation of the formation of the reproductive organs. No species developed any seed- stalks in the course of the first experiment.

6. Number of stomata per sq. mm.

On the abaxial epidermis of all the seven species investigated there are about 40–50% more stomata than on the surface, and this ratio was hardly changed by the treatment. The stomata numbers of lettuce and spinach were

Fig. 1. *Lactuca s.* var. *cap.* convar. „King of May", May—June experiment, abaxial epidermis (x200)

1. uncovered control
2. colourless foil cover
3. blue foil cover
4. yellow foil cover
5. red foil cover

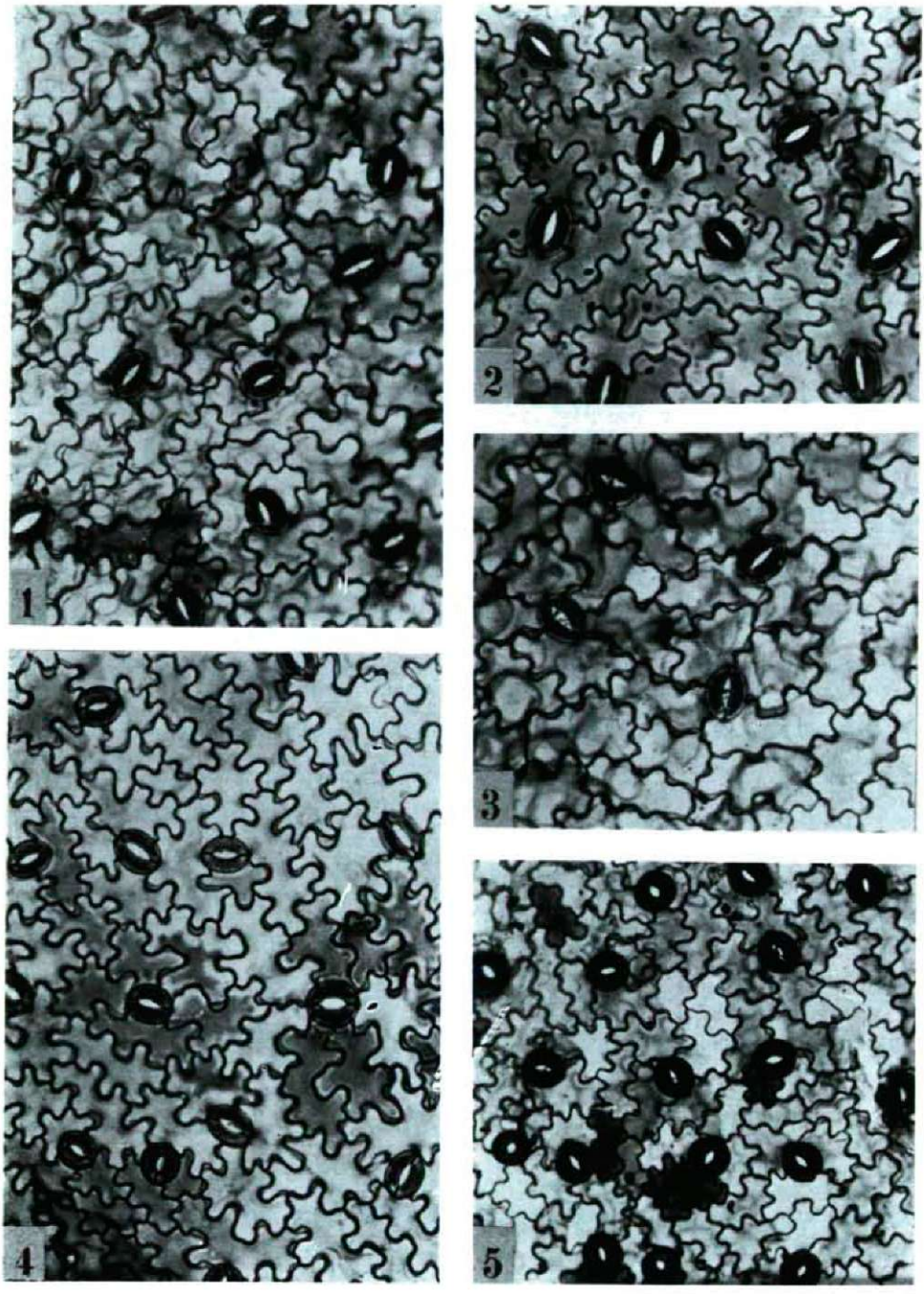

Fig. 1

increased by a higher intensity of illumination, the maximum value being given by the control. This effect can be observed in the case of sorrel, too, but the most stomata appear for the colourless variants. The stomata number is increased by raising the proportion of short-wave rays if it is connected with a high energy level. For „King of May", the spectral effect is manifested after enhancement of the red rays, with a high value exceeding even a 0,1% significance level (Fig. 1). Sorrel, however, responds more sensitively to the rays of short wave-length in the case of a low intensity of illumination, because after the colourless variants the epidermis of the blue variants is the most densely stomatized (Table 6).

In the June–July experiment, the most stomata developed under yellow foil. No spectral effect was manifested; for instance, there was no significant difference between the plants under red and blue foils. For spinach, similarly, an increase in the proportion of the yellow-orange-red spectral ranges increases the frequency of stomata.

The specific effect of the red foil must be emphasized, as the stoma number in 1 sq. mm. of the red-covered individuals of Lactuca, that has a small leaf surface, was very high in May–June (Fig. 1). In July, on the other hand, the individuals of largest leaf surface grew under red foil but the stoma number per unit surface was the lowest (Table 7).

Table 6. May—June experiment

variant		*L. sativa* var. *cap.* convar.			*S. oleracea* var. *glabra* convar.			*R. acetosa* var. *hort.* convar.
		„King of May"	Buda	Sorok-sár	Eskimo	Matador	Viro-flay	Large-leaved
uncovered control		123	167	201	195	265	152	76
colourless		140	153	151	143	181	169	108
yellow		148	139	155	157	156	106	87
blue		150	142	162	159	67	95	92
red		259	127	169	156	203	122	67
S. D. %	0.1	26	6,3	8,4	10,5	46,5	13,1	3,3
	1	20	4,9	6,6	8,3	37	10,3	2,5

Table 7. June—July experiment

variant		*L. sativa* var. *cap.* convar.			*S. oleracea* var. *glabra* convar.		
		„King of May"	Buda	Sorok-sár	Eskimo	Matador	Viro-flay
uncovered control		160	149	180	168	259	165
colourless		155	142	133	164	194	187
yellow		162	161	140	216	225	193
blue		128	122	114	—	183	143
red		127	105	124	169	206	162
S. D. %	0.1	6,7	6	7,7	8,8	12,8	10,3
	1	5,3	4,5	6,1	6,9	9,9	8,1

7. Thickness of the leaf-blade

For lettuce and sorrel, the quantity of the assimilating tissue and, at the same time, the actual thickness of the leaves are the largest (exceeding the control) under colourless and yellow foils (Fig. 2). In the Figure, the joint mean values of the three species in the May–June experiment are given.

For the spinach species, not only the yellow foil cover, but also the red one yielded thicker leaves. The enhanced red spectral range increased the thickness of the leaf in a linear way for spinach. In the case of lettuce and sorrel, there is no spectral effect on the optimum curve (Fig. 2).

In the June–July production, the plants developed in full sunshine and under yellow foils have the thickest mesophyllum (Fig. 3).

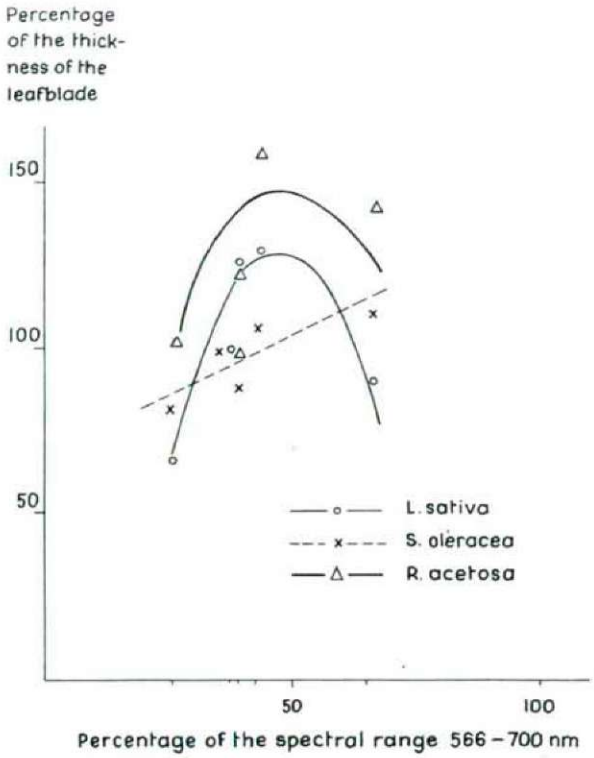

Fig. 2. Thickness of the leaf-blade as compared to the uncovered control

Fig. 3. *Spinacia o.* var. *glabra* convar. Viroflay June—July experiment, palisade and spongious parenchyma (x200)

1. uncovered control
2. colourless foil cover
3. blue foil cover
4. yellow foil cover
5. red foil cover

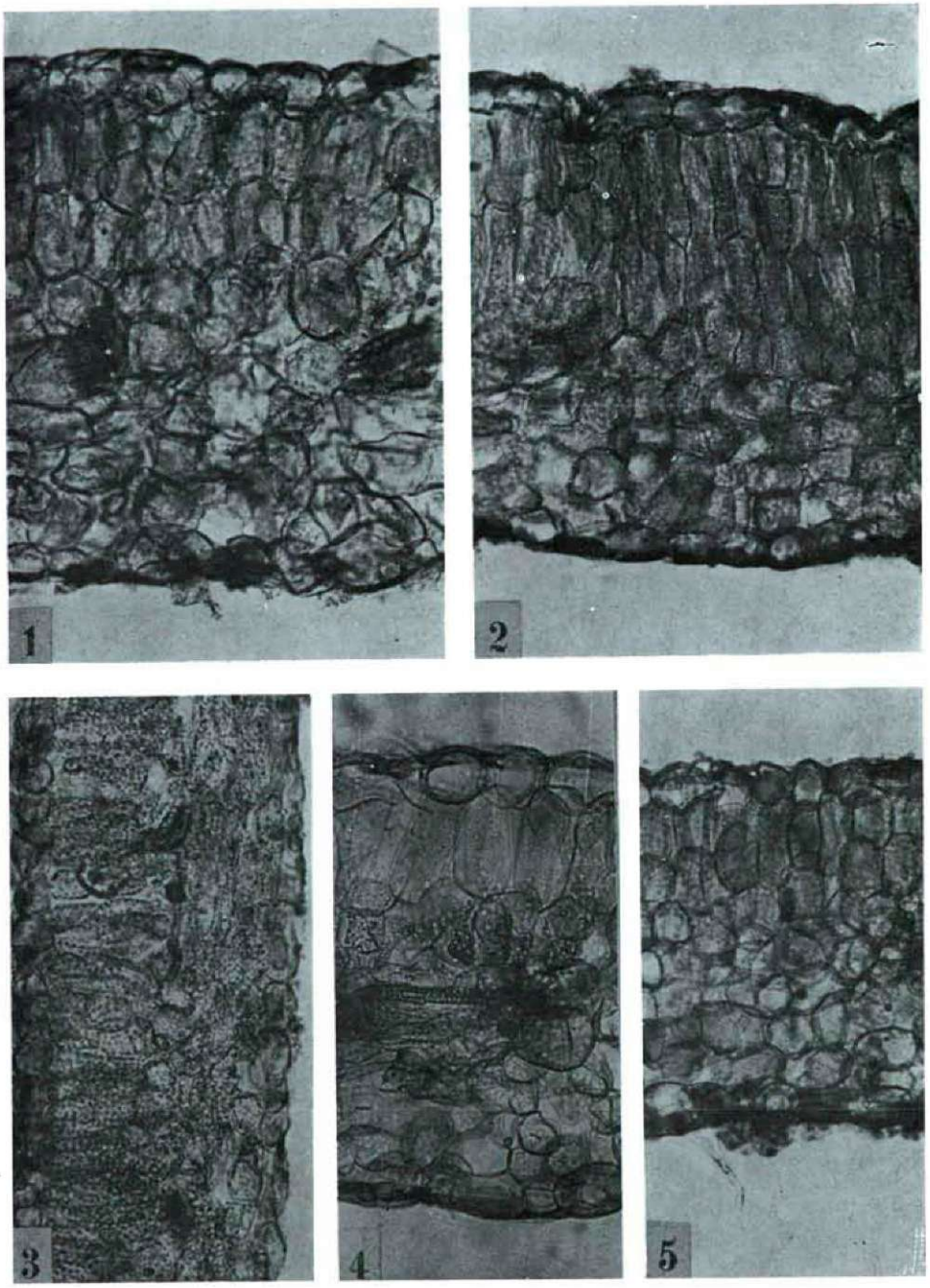

Fig. 3

8. Palisade parenchyma

The tissue of the *Lactuca* and *Rumex* palisade parenchyma is unilinear and the number of layers was not affected by the various covers. For *Spinacia,* in the case of a high intensity of illumination (control and colourless foil) cover even a three-layer palisade tissue developed. The other treatments resulted in a palisade parenchyma of double cell lines (Fig. 3). For *Spinacia,* the most developed palisade was brought about by different foil covers for different species (Viroflay – control, Matador – colourless foil, Eskimo – red foil). In the June–July production, a rise in the energy level of the light increases the development of the palisade tissue. For spinach, this effect is manifested in the change in layer number, while for lettuce it appears only in the lengthening of the cells. No spectral effect was shown: the thickness of the palisade tissue was reduced by the high proportion of both the blue and red spectral ranges. Sorrel is the most sensitive to the rays of short wave-lengt. The diameter of its palisade cells perpendicular to the surface is 90 μ in the control plants, that is one-third of the leaf cross-section. In comparison, in plants under blue foil this falls to 65 μ, the difference exceeding the 0,1% significance level.

9. Spongious parenchyma

In the earlier experiment the (colourless, yellow) foil covering was more favourable, but in June–July the control individuals had the thickest spongious parenchyma. For spinach, the enhancement of the long-wave under the red foil of low intensity illumination brought about a spongious parenchyma that athained and even exceeded the development of the control parenchyma (Fig. 4 illustrates the results of the May–June experiment, according to species, as a function of the proportion of the red spectral range).

The layer number of the spongious parenchyma changes, too, under the different foils. The control lettuce and its colourless and yellow variants have 7 to 8 cell-layers; under blue and red foils, however, there are 5 to 7 layers. For the blue variants of spinach, the layer number of the spongious parenchyma varies between 4 and 6, and the thickness of the spongy tissue is also the smallest here. The change of the layer numbers between 5 and 8 in the course of the treatment is accompanied by a considerable increase in the direction of the enhanced red spectral range (Fig. 4).

In the June–July experiment, too, the thinnest spongy tissue was a result of the blue foil coverage. For spinach, the thickness of the spongious parenchyma decreases in a linear way as a result of an increases in the proportion of the energy in the violet-blue-green spectral range. In the lettuce and sorrel leaves, the amount of the spongious parenchyma changes according to an optimum curve as a function of the enhancement of the red spectral range (Fig. 4).

10. Phloem tissue

In the May–June experiment, both the smallest and largest intensity of illumination developed only a small number of phloem elements. For lettuce, there is no spectral effect as there is hardly any difference under blue and red foil covers. The largest amount of phloem was brought about by yellow foil (two or three times more than for blue and red variants). For the sorrel and spinach species, the phloem part of the conducting tissue was enlarged

to the highest degree by the red foil covering; for Eskimo it is nearly twice that in the control (Fig. 5). The smallest phloem was produced by a low energy level and by the most enhanced violet-blue-green spectral range.

In the June–July experiment, we again meet the increased development of the phloem elements as a result of enhancing the red spectral range. For spinach this is manifested more intensely than for lettuce, because it produced an amount of phloem tissue that exceeded all the other variants. For lettuce,

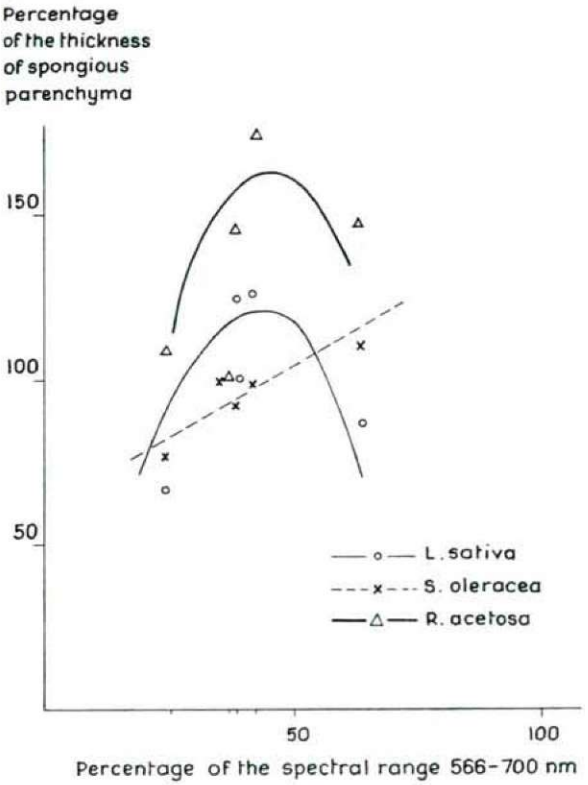

Fig. 4. Thikness of the spongious parenchyma apared to the uncovered control

there is a great difference according to species. Our results confirm the findings of JANKOVICH (1956), who lays down as a rule that on proceeding from blue towards red light, the amount of conducting tissue keeps on growing.

Fig. 5. *Spinacia o.* var. *glabra* convar. Eskimo May—June experiment, cross-section of the main vascular bundle (x200)
1. uncovered control
2. red foil cover

Lactuca s. var. *ccp.* convar. Buda, June—July experiment, cross-section of the main vascular bundle (x200)
3. uncovered control
4. blue foil cover
5. red foil cover

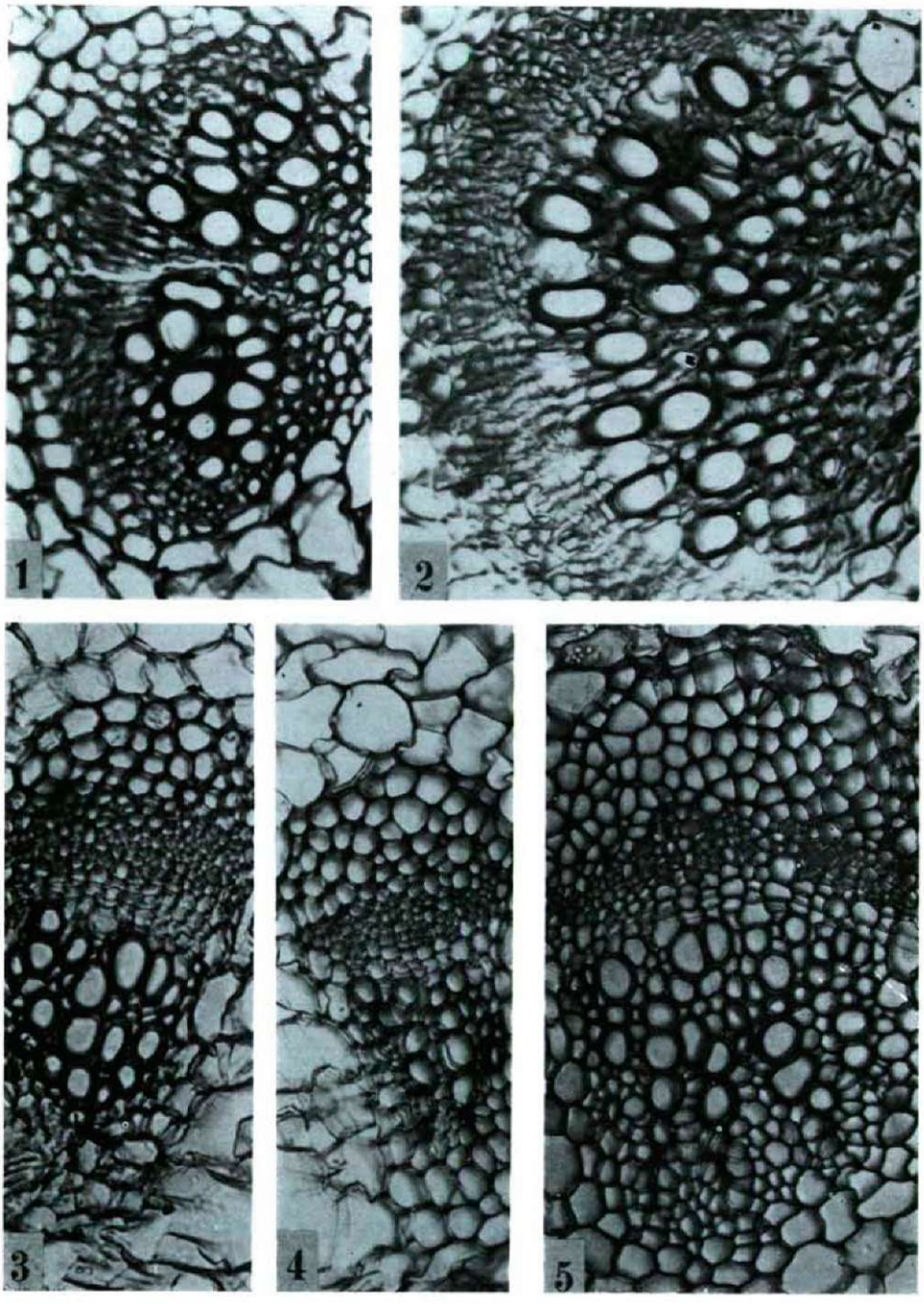

Fig. 5

11. Ligneous part

The lettuces grown in the period May–June developed three or four times more ligneous elements under colourless and yellow foils than under blue and red foils. It is shown by the optimum curve that the spectral effect has no influence on the ligneous part, because there is no considerable enhanced (Fig. 6 – shows the change in the amount of ligneous elements, in the average of three species each, in the period of the May–June production).

For sorrel, on the increase of the light energy, the ligneous part decreases, being the smallest for the control. The spectral effect is expressed in that an increase in the proportion of the red spectral range produced a more developed ligneous part (Fig. 6).

For spinach, as compared to the control, the development of the ligneous part was reduced by the colourless and blue covers, but considerably increased by the yellow and red covers (Fig. 5).

In the June–July experiment, the growth of the ligneous part is of a higher degree in the direction from the blue to the red spectral range. This effect is manifested for lettuces, too.

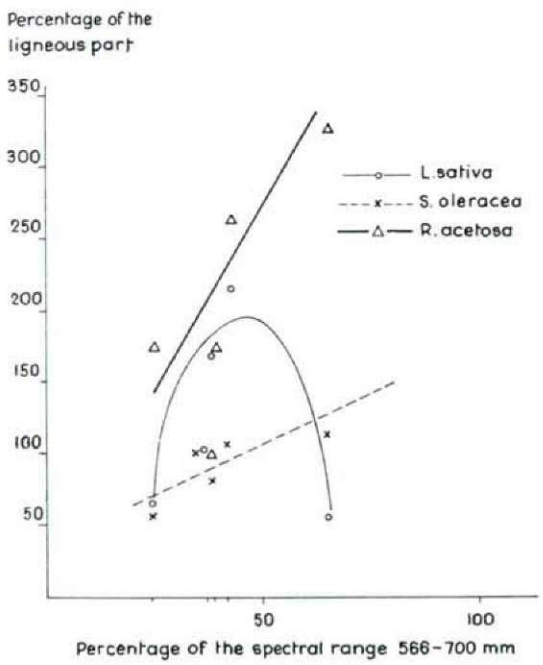

Fig. 6. Development of the ligneous part of the main vessel as compared to the uncovered control

Summary

We have investigated the morphological, phenological, and leaf-anatomical changes in cabbage lettuce, spinach, and sorrel species as functions of the intensity of illumination and spectral energy distribution. The field experiments

were carried out in May–June and June–July. Colourless, blue, yellow and red perforated foil covers were applied.

Epidermis preparations were made by maceration and sectioning of the leaves, and the quantitative properties of the tissue elements were evaluated by biometric methods. The treatments induced significant changes in a great part of the features investigated.

1. The effect of the intensity of illumination can be summed up as follows:

In the May–June period, increase in the light energy leads to the parallel increase of the fresh leaf weight of cabbage lettuces, the surface of the leaf, the leaf number and the stem length. In the June–July production, on the other hand- the higher temperature being, associated with less precipitation – the leaf surface, leaf production and stem length of cabbage lettuces growing in full sunshine are less.

The correlation between the number of stomata per unit area and the increase in energy level is generally positive.

In the June–July experiment, for all the three cabbage lettuces, there was a positive correlation between the increase of the intensity of illumination, the length of the palisade parenchyma cells, and the thickness of the leaf, as well as the amount of lignaceous elements. Further in the case of „King of May", the effect of the light intensity is proved by the stomata number and the tissue thickness of the spongious parenchyma tissue increasing. In the case of Spinacia, the layer thickness and layer number of the palisade parenchyma similarly increase in the direction of a higher energy level. The leaf surface of sorrel was increased by the reduced light intensity, but the leaf number was lower.

2. The spectral effect of the light:

The role of the spectral composition is shown by the fresh leaf weight, the increase in the thickness of the leaf-blade, the increase of the leaf surface, and a major lengthening of the cells of the palisade parenchyma. The thickness of the leaf-blade and, closely connected, the amount of the spongious tissue in the lettuce and sorrel leaves increase according to an optimum curve, but for spinach in a linear way, together with the increase in the proportion of the red spectral range.

A different change is also induced, in the three species by enhancing the yellow-orange-red spectral range (primarily in the ligneous part). In the leaves of sorrel and spinach a linear growth appears, while in lettuce the change takes place according to an optimum curve.

From among the phenological properties, the shooting of the seed-stalks of spinach is inhibited by red light.

The stomata number per unit area is very high on enhancing the proportion of the violet-blue-green spectral range. In addition, the increase in the proportion of the short-wave rays decreased the surface of the leaf-blade, the

leaf thickness and the amount of conductive tissues (particularly of the phloem) and inhibited the lengthening of the palisade cells. The mesophyllum tissue of the blue variants are therefore very undeveloped.

3. Taking into consideration some practical points of view, the following conclusions can be drawn:

a) In the earlier production of lettuce, the features determining the „production" (leaf number, size of leaf-blade, thickness of the leaf) were mostly increased by yellow and colourless foil covers. The yellow foil also has a favourable influence on the anatomical properties (development of mesophyllum, conductive tissue).

In the later (June–July) production, on the basis of the above points of view, a red foil too can be taken into consideration.

b) For spinach, the production is increased by the red and yellow foils in both experimental periods.

c) The fresh weight of sorrel (leaf number, leaf size) is the highest in full sunshine. However, taking into consideration the anatomical properties (mesophyllum, conductive tissue system), too, yellow and red foil covers can also be taken into consideration.

d) In the case of these garden plants, a blue foil cover is not advisable as the production of leaves is markedly decreased.

References

DULLFORCE, M. M. (1969): Light temperature balance vital in heading timing. — Grower, London, *72*, 121—123.

FLECHTER, R. A.—PETERSON, R. L.—ZALIK, S. (1965): Effect of light quality on elongation, adventitious root production and the relation of cell number and cell size to bean seedlings elongation. — Plant Phys. *40*, 541—548.

FORTANIER, E. L. (1954): Some observations on the influence of spectral regions of light on stem elongation, flower-bud elongation, flower-bud opening and leaf movement in *Arachis hypogea*. — Medelel. Landbowhoogesch. *54*, 103—114.

HORVÁTH, I. (1965): A fény színképi összetételének növényökológiai szerepe (Plant-ecological role of the spectral composition of light). — Acad. Doct. Diss. Gödöllő.

HORVÁTH, I.—GULYÁS, S.—LÁSZLÓ, G. (1966): A fény színképi összetételének hatása a *Phaseolus vulgaris* és *Glycine soja* szárának szöveti szerkezetére — II. (Effect of the spectral composition of light on the tissue structure of the stem of *Phaseolus vulgaris* and *Glysine soja* — II). Hungarian Plantanatomical Symposium Budapest, 3/5.

HORVÁTH, I.—KOLTAY, L. (1967): A fény színképi összetételének hatása a szárazanyag-produkcióra és lokalizációjára. — Mezőgazdasági Kari Közl. (Effect of the spectral composition of light on the production and localization of dry matter. — Publ. of the Agricultural Fac.). Gödöllő, *141*.

JANKOVICH, V. (1956): Uticaj vostacko spetleski na porast i anatomija nekih biljaha. — Arch. Poljopriv Nauke. *9*, 65—75.

LÁSZLÓ, G.—SZALAI, M. (1964): A fény színképi összetételének hatása a borsószár szövettájainak arányára. — Tud. Diákköri Dolg. (Effect of the spectral composition of light

on the ratio of tissue regions in the pea stem. — Peper in the Sci Stud. Circle). — Gödöllő.

MATTEI, F. (1967): Effetto dell' ombregiamento sulla lottuga (*Lactuca sativa* L.). — Riv. Ortoflorofruttic. Ital. Firenze *92*, 206—215.

MCCLEDON, I. H. (1962): The relationship between the thickness of decidous leaves and their maximum photosyntetic rate. — Amer. I. Bot. *19*, 320—322.

NUERNBERGK, E. L. (1966): Mesterséges fény és a növények (Artificial light and plants). — Agric. Publ. House. Budapest.

PARKER, M. W.—HEDRICKS, S. B.—BORTHWICK, H. A.—WENT, F. W. (1949): Spectral sensitivities for leaf and stem growth of etiolated pea seedlings and their similarity to action spectra for photoperiodis m. — Amer. Journ. Bot. *36*, 194—204.

PÁL, M. (1966): A fény színképi összetételének hatása a kultúrnövények morfológiai-fenológiai tulajdonságaira és szárazanyag produkciójára. — Egyet. Dokt. Ért. (Effect of the spectral composition of light on the morphological-phenological properties and dry matter production of the cultivated plants. — Univ. Doct. Diss.). — Gödöllő—Nyíregyháza.

SENEBIER, I. (1785): Physikalisch-chemische Abhandlungen. 2.

SIMON, MRS.—SZEGEDI, J. (1970): Különböző színű fóliasátrak alatt nevelt borsók szöveti tulajdonságaira (Effect of foil coverages of different colours on the tissue properties of colours — II.). — Bot. Közl. *57*, 292—302.

SIMON, MRS.—SZEGEDI, J. (1969): Különböző színű fóliatakarás hatása a borsólevél szöveti tulajdonságaira (Effect of foil coverages of different colours on the tissue properties of pea leaves). — J. of Agric. Univ. Gödöllő, 249—264.

SOMOS, A. (1967): Zöldségtermesztés (Market gardening). — Agricult. Publ. House. Budapest, 36—37.

TIMIRYAZEV, K. (1875): Ob usvocnii sveta rasteniyani — Peterburg.

VINCE, D. (1956): Studies of the effects of light quality on the growth and development of plant. — Hort. Sci. *31*, 16—24.

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EFFECT OF ACTINOMYCIN D AND 2-THIOURACIL ON THE ELONGATION OF OAT COLEOPTILES INDUCED BY DIFFERENT PHENOLIC ACIDS

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Introduction

It is known that high concentrations of some phenolic acids occurring naturally in plants inhibit and lower, physiological concentrations of them promote the growth. It is also established that these compounds show an interaction with indoleacetic acid (IAA) as antagonists or synergists (VARGA, 1957; VARGA és KÖVES, 1959; TOMASZEVSKI, 1964; KÖVES, 1965). The possible basis of the interaction is the promotion or inhibition of the IAA-oxidase, however, this explanation can be not quite satisfying in some cases (KÖVES, 1965; KÖVES, SIROKMÁN és MILASSIN, 1972).

Our previous examination showed that low concentrations of some phenolic acids enhance the incorporation of 14–C-leucine into the protein fraction of bean hypocotyl segments and this effect of these compounds is similar to that of the phytohormones.

These results provide a possibility to suppose that – similarly to the hormones – one of the essential parts of the action of phenolic acids in the growth is the regulation of protein synthesis. In present paper the authors wish to investigate how specific inhibitors of protein synthesis influence the effect of some phenolic acids on the elongation of coleoptile segments.

Materials and Methods

The compounds applied in the experiments were: o-coumaric-, salicylic-, gallic-, p-oxibenzoic-, ferulic- and chlorogenic acids in concentrations 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} M.

Twentyfive segments of *Avena coleoptile* (5 mm) were floated as test-material in a solution containing different combinations of Actinomycin D (1, 10 and 20 μg/ml) or 2-thiouracil (10^{-3}, 10^{-4} and 10^{-5} M) with 10^{-4}, 10^{-5} and 10^{-6} M phenolic acid, up to 5 ml of phosphate-buffer pH 6,5. The incubation took place for 24 hours 24 °C, followed by measurement of the elongation of coleoptile segments.

Results and discussion

The growth-promoting effect of the applied phenolic acids is the highest at 10^{-6} M and it takes about 20%. Almost in all combinations examined, 1 μg/ml of Actinomycin D was sufficient to inhibit the growth induced by phenols but in some cases the inhibition is significant only at 10 μg/ml of Actinomycin D concentration, because of the high value of the standard devi-

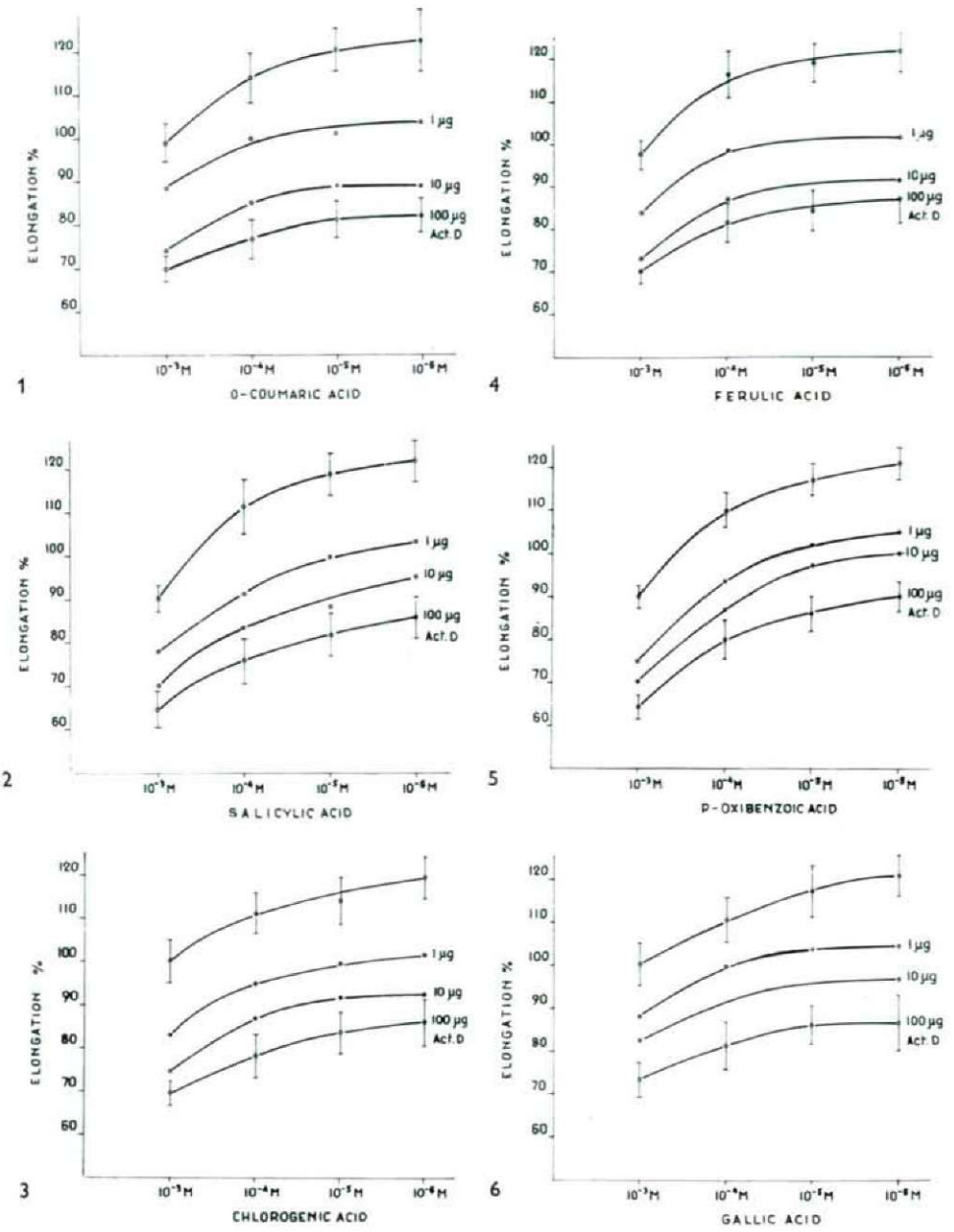

Fig. 1—6. Effect of Actinomycin D in different concentrations on the elongation induced by o-coumaric- (1), salicylic-, (2) chlorogenic-, (3) ferulic-, (4) p-oxi-benzoic- (5) and gallic acid (6).

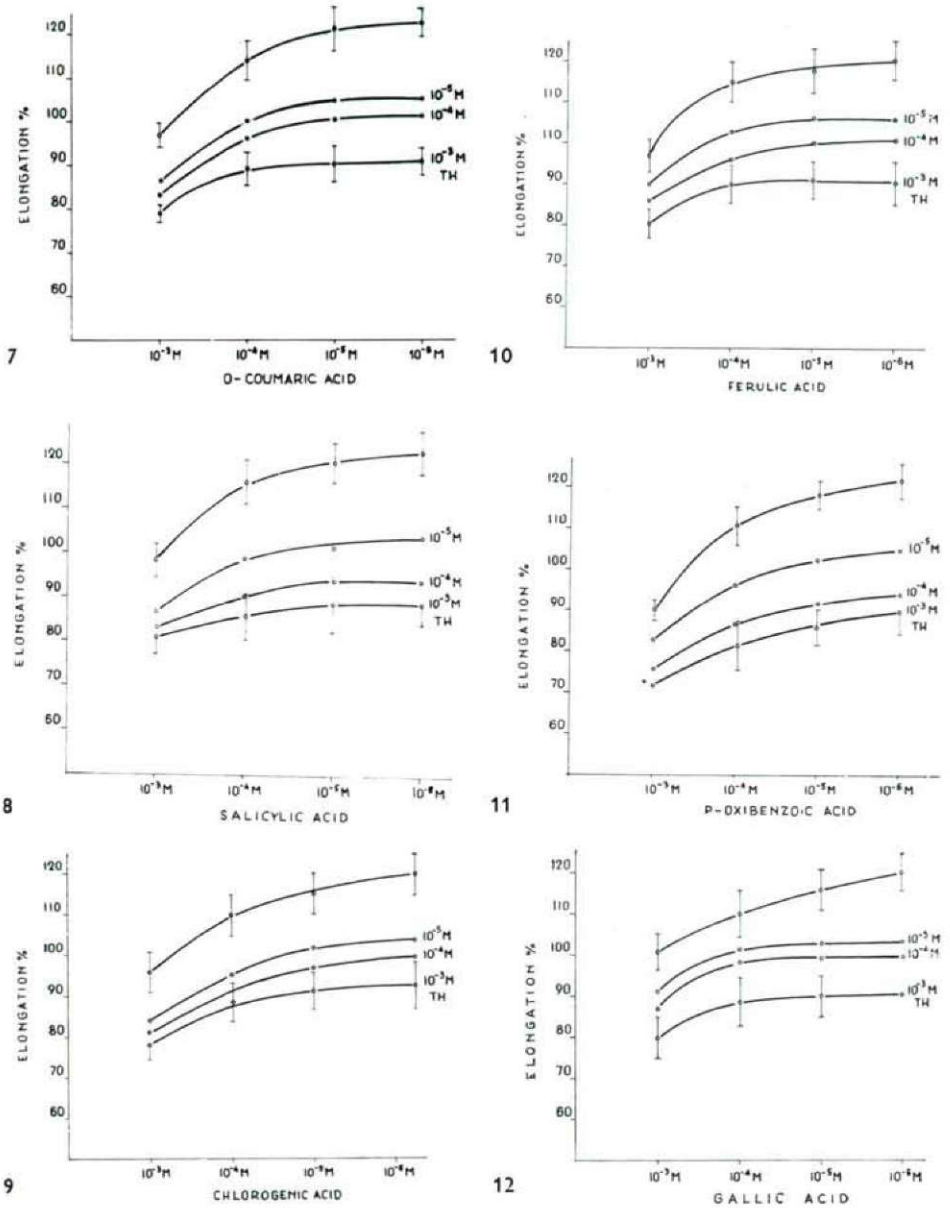

Fig. 7—12. Effect of 2-thiouracil in different concentrations on the elongation induced by o-coumaric-, (7) salicylic-, (8) chlorogenic-, (9) ferulic-, (10) p-oxi-benzoic- (11) and gallic acid (12).

ation. The results obtained at higher concentrations of Actinomycin D show that the inhibitor decreases not only the growth induced by phenols, but also the normal growth measured in the control segments.

2-Thiouracil at 10^{-4} M concentration has some inhibiting activity while at 10^{-3} M inhibits the growth induced by phenolic acids significatly in all of the experiments.

In the investigations described above, it is proved that the growth promotion induced by phenolic acids can be prevented by inhibitors of protein synthesis. The phenolic acids in lower concentrations have an effect resembling that of the phytohormones in this respect too. This effect is however, slighter than that produced by the phytohormones. In some cases the auxin synergisms can be explained by this properties of the phenolic compounds.

The result obtained complete the knowledges on the mode of action of phenolic acids, providing some informations about the effects produced by its physiological concentrations.

Summary

Growth promotion induced by six naturally occurring phenolic acids (o-coumaric-, salicylic-, ferulic-, p-oxi-benzioc-, chlorogenic- and gallic acid) can be inhibited by 10 μg/ml Actinomycin D and 10^{-4} M 2-thiouracil. Increasing the concentration of the inhibitors with one magnitude the normal elongation of the coleoptils can be inhibited too. In addition to the results of our earlier investigations, present data also show that the effect of the physiological concentrations of the above mentioned phenolic compounds on the protein metabolism resembling that produced by phytohormones.

References

KÖVES, E. (1965): Adatok a természetesen előforduló fenolkarbonsavak növekedésszabályozó hatásmechanizmusához (Data for growth regulating mechanism of naturally occurring phenolic acids). — Thesis for C. Sc. Biol. Szeged.

KÖVES, E., SIROKMÁN, F., MILASSIN, M. (1972): Connection between the Growth Regulating Action of Plant Phenolic Compounds and the Protein Synthesis. — Z. Pflanzenphysiol. *67*, 370—372.

TOMASZEVSKI, M. (1964): The mechanism of synergistic effects between auxin and some natural phenolic substances. — Colloq. Intern. Centre Natl. Rech. Sci. Paris *123*, 335—351.

VARGA, M. (1957): Növekedésgátló anyagok papírkromatográfiás vizsgálata, különös tekintettel a húsos termésekre (Paperchromatographic examination of growth inhibiting substances with special respect to fleshy fruits). — Thesis for C. Sc. Biol., Szeged.

VARGA, M., KÖVES, E. (1958): Vorkommen, Verteilung und quantitative Veränderung des β-Inhibitors in den einzelnen Organen der Bohnenpflanze. — Naturwiss. *45*, 468—469.

VARGA, M. (1968): Az indolecetsav és gibberellinsav együtthatása a szármegnyúlásban (Interaction of IAA and Gibberellic Acid on the Stem Elongation). — Thesis for. D. Sc. Biol., Szeged.

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THE EFFECTS OF THE CULTURE MEDIUM AND LIGHT ON THE PIGMENT-SYNTHESIS AND SPORULATION IN ASPERGILLUS AND PENICILLIUM GENERA*

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Summary

A study was made of the pigment-synthesis and sporulation of *Aspergillus repens, Penicillium notatum* and *Penicillium purpurogenum* on

1. Czapek-Dox,
2. Czapek-Dox supplemented with microelements,
3. potato agar, and
4. modified Czapek-Dox

culture media, illuminated with white, blue, green, orange-yellow, red and periodic light, or in the dark.

1. The experimental results showed that *Penicillium notatum* does not exhibit photosensitivity. The pigmentation and sporulation of *Penicillium purpurogenum* and *Aspergillus repens* are significantly inhibited by blue light, and to a lesser extent by green and orange-yellow light, while they are enhanced by red light and by darkness compared to the white light control.

2. The sporulation of *Penicillium notatum* and *Aspergillus repens* are delayed by blue light, accelerated by the dark, and unaffected by green, orange-yellow and red lights compared to the white control. There is no difference for *Penicillium purporogenum.*

3. In red illumination and in the dark the length of the conidiophores of *Aspergillus repens* decreases.

4. The vitality of the spores from the younger zones of the colony is greater than that of the spores in the older zones.

5. As regards the culture medium effects, Zn delays the pigmentation and sporulation in all three species, most strongly in *Penicillium purpurogenum,* and least in *Aspergillus repens.*

6. The zonation is inhibited by the presence of Cu in the culture medium, and enhanced by that of Mn. Zn apparently plays no role in the development of the zonation.

7. With regard to the nitrogen sources, the species studied utilize best the NH_4^+, less well the NO_3^-, and slowest of all the nitrogen of urea.

* Part and thesis from the diploma-paper of Miss MÁRIA TÓTH.

Introduction

It is generally characteristic of the entire vegetable kingdom that the reproductive organs are more richly pigmented than the vegetative organs. In the higher-order plants the varied display of colours of the flowers is provided by the anthocyanins, the various flavone derivatives and the carotenoids. Colonies of sporulating fungi are just as rich in colour, and differently pigmented from the mycelium. The question arises of whether there is a correlation between the individual stages of the sporulation, the colour contents of the pigments and their intensities. It has long been known that a whole series of pigments can be found in the various fungi. However, far less is known of the varied forms of the pigments or of their physiological roles than, for example, in the higher-order plants, in spite of the fact that many papers have been published on this theme (BESSEY, 1904; CARLILE, 1965; EBERHARD et al., 1961; NARASIMHACHARI, 1963). Even less in known of the connection between the appearance of the pigments and the sprorulation, which also justifies the study of these problems.

The strains of the genera *Aspergillus* and *Penicillium* selected for the experiments are noted for being rapidly and easily cultivated, for their abundant sporulation, and for their variably coloured pigments. On the basis of these three aspects, preliminary experiments on 10 *Penicillium* and 20 *Aspergillus* strains led to the selection of the following species:

Penicillium purpurogenum	(787)*
Penicillium notatum	(190)
Aspergillus repens	(–)

In accordance with the aims of the experiment, a study was made of:

a) The effects exerted on the sporulation and the pigmentation accompanying the sporulation by culture media differing essentially from each other in composition.

b) The effects exerted on the sporulation and pigmentation by the individual monochromatic light regions for strains cultivated on the same culture medium.

c) The intensity of the sporulation in both cases, i.e. as a function of the culture medium and the light. Our results were then compared with previous literature findings.

The reality of the problem raised is supported by a number of literature findings. Neurospora crassa conidium never contains carotinoid if it grows in the dark, but light promotes carotinoid synthesis in the mycelium (ZALOKAR, 1954).A number of literature data indicate that the photo-effect also depends on composition of the culture medium (CARLILE, 1965; MUNTANJOLA et al., 1968). For example, when *Penicillium clavigerum* is cultivated on malt agar it is insensitive to light, and grows with the same intensity in light or darkness; however, in 12-hour illumination daily on Czapek agar the rate of growth remains the same, whereas in 24-hour illumination it decreases. On the other hand, *Penicil-*

* The numbers in brackets denote the strain cultures maintained in the Viticultural and Oenological Research Institute.

lium claviforme requires light at the beginning of its development, until the mycelia have attained a length of some mm, but after this it becomes, apparently insensitive to the illumination (CARLILE, 1965). In contrast, *Penicillium isariforme* is sensitive to light throughout its entire life (CARLILE et al., 1962). These examples show that even species belonging to the same genus react in totally different ways to light, at times depending on their state of development, at others on the composition of the culture medium; thus, the sensitivity or insensitivity of the species to light can not be correlated with the kinship. It is unfortunate that extremely few literature data can be found with regard to the effects of light and culture medium on the sporulation. In the present paper new approaches are made and new relationships are sought, and hence there are few possibilities to compare our results with literature data.

Not only the pigmentation, but also the sporulation is affected by the nature of the light and the composition of the culture medium. The development, presence and absence of the fruit bodies, the spores and the conidia can be affected by light, while in addition their form and differentiation too depend on the illumination. *Aspergillus aureolatus* grows well in an appropriate nutrient medium in the dark, and the sporulation too is maximum, whereas in light the conidial formation is poorer (MUNTANJOLA et al., 1968; CARLILE, 1962). It must be mentioned, however, that the data of papers dealing with sporulation and light conditions are not only different, but in many cases contradictory, and thus it is at present not possible to give a uniform picture of this question. Several authors (FRIEDERICKSEN and ENGEL, 1960; LUKENS, 1963; MOHR, 1961) have stressed that sporulation is affected only by the short-wave region, the longer waves being ineffective.

The aim our studies is to attempt to decide between these contradictory data, and to provide extra information in this field of plant physiology, which in many respects is still unknown. It is assumed that the pigmentation is a fundamental factor, which influences not only the sporulation, but also the morphogenesis of the fungi.

Materials and Methods

1. Experimental objects

The species and strains used in the experiment were made available by the National Viticultural and Oenological Research Institute. They were maintained on potato agar culture medium in test-tubes, under a protective layer of paraffin oil, and always these accurately determined strains were used in the transoculations for multiplication.

2. Culture media

The following culture media were used to study the effects of the media:

a. Czapek—Dox solid culture medium, to which the sugar was added only before the final sterilization.

b. Czapek—Dox solid culture medium supplemented with microelements in the following four variations:

(1)	Basal nutrient medium (a) + $MnSO_4$	0.0025	g
(2)	Basal nutrient medium (a) + $ZnSO_4$	0.0025	g
(3)	Basal nutrient medium (a) + $CuSO_4 \cdot 5H_2O$	0.0025	g
(4)	Basel nutrient medium (a) + $MnSO_4$	0.001	g
	+ $ZnSO_4$	0.001	g
	+ $CuSO_4$	0.001	g

c. Potato agar culture medium (SZALAI and FRENYÓ, 1962).
d. Modified Czapek—Dox culture medium in three variations, containing 3 g $(NH_4)_2SO_4$, 3 g NH_4NO_3 or 3 g urea in place of $NaNO_3$ as source of nitrogen.

The culture media were freshly prepared, and after adjustment to the appropriate pH 30 ml of culture medium was poured into Petri dishes 10 cm in diameter.

After sterilization the culture media were stored for 3—4 days in thermostats at room temperature, and only those were used for inoculation which remained sterile. Under the usual sterile conditions, infection-free cultures were attained in 80% of the transoculations.

3. Cultural procedure (incubation)

The cultural conditions were varied according to the aims of the experiment. The cultures were placed in thermostats, kept in the dark, and developed at 26—28 °C (± 0.2 °C). In order to study the effect of light, the cultures were placed in a climatic chamber the temperature of which was 22—23 °C (± 1 °C).

The climatic chamber could be illuminated with light enriched in monochromatic light by the use of exchangeable white, red, orange-red, green and blue discharge tubes.

4. Study methods

The pigmentation and sporulation in the cultures were observed for 12 days following the transoculation.

Data recorded included the number, dimensions and development of the pigmentation of the concentric rings (zonation).

The development of the sporulation was followed in part with the naked eye, and in part by cytoscope, and was supplemented with microscopic measurements. For the exact performance of the measurements a squashed preparation was formed in the usual way (SÁRKÁNY and SZALAI, 1966), and the diameters of the spores, the lengths and diameters of the conidiophores and the diameters of the vesicles were measured with an ocular microscope.

Experimental results and observations

One aim of the work was to determine the changes induced in the sporulation of the fungi by the composition of the culture medium and the nature of the light. Attention was paid to:

a) the beginning and course of the pigmentation of the mycelium;
b) the time and course of the sporulation;
c) the structure of the zones formed during the sporulation.

The pigmentation of *Penicillium purpurogenum* on Czapek-Dox agar in various monochromatic lights

1. In white light the pigmentation of the mycelium begins visibly on the lower side of the culture medium on the fifth day, and proceeds elliptically outwards. The mycelial mass is wine-red in colour, and the pigment diffusing out into the culture medium is a vivid purple. The sporulation begins in the centre of the colony on the eighth day and proceeds outwards in rings, and on the tenth day the entire colony is sporulating (Table 1). The zonation (Fig. 1) is not definite (it is indistinct), and proceeding autwards fromt the centre: dark grey (0.6 cm) → greenish-grey (1.5 cm → light brown (0.5 cm) greyish-brown (0.6 cm).

2. In blue light the pigmentation in the mycelium begins on the sixth day, but the characteristic purple colour develops more slowly than in white light. Only an extremely small amount of pigment diffuses out of the mycelium into the culture medium, and therefore this is a pale pink colour. The sporulation in blue light begins only on the tenth day (Table 1). The sporulation is accom-

panied by zonation (Fig. 1). Seven zones develop: dark grey (0.2 cm) → brownish-grey (0.3 cm) → black (0.3 cm) → brownish-grey (0.4 cm) → dark grey (0.6 cm) → brownish-grey (0.3 cm) → light grey (0.3 cm).

3. In green light the pigmentation in the mycelium begins on the fifth day, with a vivid purple colour. The diffusion out of the pigment into the culture medium is less significant than in white light, but stronger than in the culture grown in blue light. The sporulation begins on the ninth day (Table 1). Ring formation occurs (Fig. 2), and four zones develop: dark grey (0.6 cm) → brownish-black (0.7 cm) → grey (0.8 cm) → greenish-grey (0.9 cm).

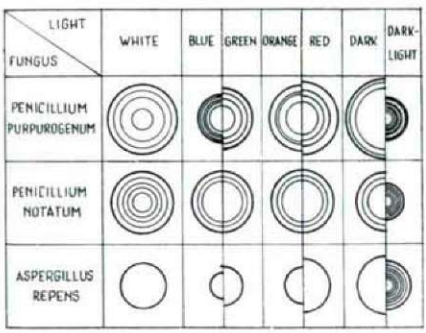

Figure 1. Effect of light on the formation of the sporulation rings

4. In orange-red light the pigmentation in the mycelium begins on the fifth day with a wine-red colour, which diffuses out abundantly into the culture medium. The sporulation begins on the ninth day, the zonation is insignificant and the colour differences and zone boundaries are not marked (Table 1). There are four zones (Fig. 1): dark grey (0.5 cm) → grey (0.8 cm) → dark grey (0.9 cm) → grey (0.8 cm).

Table 1. Zonation, pigmentation and sporulation of *Penicillium purpurogenum* in different monochromatic lights

Light	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
White	4	5	8
Blue	7	6	10
Green	4	5	9
Orange	4	5	9
Red	4	5	8
Dark	3	5	7
Periodic	12	6	9

5. In red light the pigmentation of the mycelium begins on the fifth day, and pigments diffuse out into the culture medium. The sporulation begins on the eighth day, i.e. earlier than in cultures illuminated with other monochromatic lights (Table 1). The zonation during the sporulation is weak (Fig 2).

Four zones develop: greenish-grey (0.7 cm) → brownish-grey (0.6 cm) → dark grey (0.9 cm) → brownish-grey (0.9 cm).

6. The pigmentation in the mycelium of a colony grown in the dark begins on the fifth day, and diffuses out very intensively into the culture medium. The sporulation begins on the seventh day, i.e. the earliest of all (Table 1). Three very weakly developed zones are formed, with indistinct boundaries (Fig. 1): brownish-grey (3.1 cm) → light brown (0.2 cm) → brownish-grey (0.3 cm).

7. In periodic illumination the pigmentation of the mycelium begins on the sixth day, and resembles the phenomena seen in white light. The sporulation begins on the ninth day (Table 1), the zonation is intense (Fig. 1), and there are 12 zones: brown (0.2 cm) → grey (0.2 cm) → brown (0.2 cm) → light brown (0.2 cm) → greyish-brown (0.2 cm) → light brown (0.2 cm) → brown (0.2 cm) → greenish-grey (0.2 cm) → dark grey (0.2 cm) → greenish-grey (0.2 cm) → light brown (0.2 cm) → grey (0.2 cm).

Pigmentation in *Penicillium purpurogenum* in constant white light on culture media of various compositions

1. On a Czapek-Dox agar culture medium containing Mn the pigmentation of the mycelium is already significant on the fifth day; the colour is wine-red, but only the orange-yellow colour-component diffuses out into the culture medium. The sporulation begins on the eight day. The ring formation is very

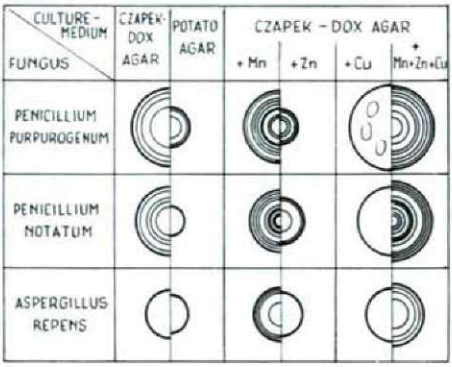

Figure 2. Effect of composition of culture media on the formation of the sporulation rings

sharp (Table 2), with nine zones (Fig. 2): grey (0.2 cm) → greenish-grey (0.3 cm) → brown (0.4 cm) → greenish-grey (0.3 cm) brown (0.4 cm) → grey (0.3 cm) → brown (0.4 cm) → light brown (0.2 cm) → brown (0.5 cm).

2. On a Czapek-Dox agar culture medium containing Zn a vivid purple pigmentation of the mycelium appears on the seventh day, and a purple colour also diffuses out into the culture medium. The sporulation can be observed only on the tenth day (Table 2). Five sharply separated zones can be distinguished in the sporulation (Fig. 2): grey (0.2 cm) → light brown (0.3 cm) → brownish-grey (0.5 cm) → pink (0.2 cm) → grey (0.6 cm).

3. On a Czapek-Dox agar culture medium containing Cu the pigmentation in the mycelium appears on the fifth day. A very strong purple colour diffuses

out into the culture medium. The sproulation appears on the eight day; rings are not formed (Table 2), but a colour difference develops on the sporulation surface, and violet-brown spots appear in the brownish-grey conidial field (Fig. 2).

4. On a Czapek-Dox agar culture medium containing Zn, Mn and Cu the pigmentation appeared on the fifth day, and the sporulation on the eighth day. The sporulation exhibits a strong zonation, but the strong zonation effect of the culture medium containing only Mn is not attained. There are seven rings (Fig. 2): greyish-brown (0.3 cm) → brown (0.3 cm) → grey (0.4 cm) → brown (0.5 cm) → brownish-violet (0.5 cm) → grey (0.6 cm) → brownish-grey (0.6 cm).

5. On potato agar culture media the yellowish-red pigmentation of the mycelium can be observed on the sixth day (Table 2), the culture medium becoming pale red. Three zones develop: greenish-grey (1.0 cm) → ligth brown (0.2 cm) → greenish-grey (0.2 cm).

Table 2. Zonation, pigmentation and sporulation of *Penicillium purpurogenum* on various culture media containing microelements

Culture medium	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
Czapek (K)	4	5	8
Czapek + Mn	9	5	8
Czapek + Zn	5	7	10
Czapek + Cu	2	5	8
Czapek + Mn + Zn + Cu	7	5	8
Potato agar	3	6	9

The pigmentation of *Penicillium notatum* on Czapek-Dox agar in various monochromatic lights

1. In white light the pigmentation of the mycelium can be observed on the third day; a pale yellow pigment is synthetized, which diffuses into the culture medium. The sporulation begins on the fifth day (Table 3). Five rings develop in the sporulation (Fig. 1): yellow (1.2 cm) → brownish-grey (0.3 cm) → light brown (0.3 cm) → dark brown (0.5 cm) → greyish-yellow (0.6 cm).

2. In monochromatic light the orange-yellow pigmentation of the mycelium begins in every case on the third day; a lemon-yellow pigment migrates out of this into the nutrient medium. The sporulation begins on the fifth day, with a yellowish-grey colour, and separates into three zones (Fig. 1). The greyish-yellow conidial field (3.2–3.5 cm) can be found at the centre, followed by a light brown ring (0.3 cm), and finally by a brownish-grey zone (0.8 cm) (Table 3).

3. In the dark the pigmentation of the mycelium is already very intense on the third day, and a very concentrated orange-yellow material diffuses out of the mycelium inti the culture medium. The sporulation appears on the fifth day, and three zones develop (Fig. 1), the colours of which are the same as

those of the cultures illuminated with monochromatic light. At the centre there is a greyish-yellow zone 3.2–3.4 cm in diameter, surrounded by a 0.3 cm thick light brown ring, with outside this a 0.8 cm thick brownish-grey ring (Table 3).

Table 3. Zonation, pigmentation and sporulation of *Penicillium notatum* in various lights

Light	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
White	5	3	5
Blue	3	3	5
Green	3	3	5
Orange	3	3	5
Red	3	3	5
Dark	3	3	5
Periodic	8	4	6

4. In periodic illumination the pigmentation begins on the fourth day, and a lemon-yellow pigment diffuses out of the orange-yellow mycelial mass into the culture medium (Table 3). The sporulation begins on the sixth day, and well separated zones develop in the conidial field. There are 8 zones (Fig. 1): light brown (1.0 cm) → brown (0.2 cm) → dark brown (0.2 cm) → greyish-brown (0.4 cm) → light brown (0.2 cm) → bluish-grey (0.3 cm).

Pigmentation and sporulation of *Penicillium notatum* in constant white light on culture media of various compositions

1. On Czapek agar culture medium containing Mn the pigmentation can be well observed on the third day; this is orange-yellow, and a lemon-yellow material diffuses out of it into the culture medium. The sporulation begins on the fifth day (Table 4), and 11 zones develop (Fig. 2): light brown (0.2 cm) → orange-yellow (0.3 cm) → greyish-yellow (0.2 cm) → brownish-yellow (0.2 cm) → light brown (0.2 cm) → brown (0.3 cm) → greyish-yellow (0.2 cm) → light brown (0.2 cm) → brown (0.4 cm) → greyish-yellow (0.3 cm) → grey (0.3 cm).

2. On Czapek agar containing Zn the pigment appears in the mycelium on the fourth day, and an orange-yellow pigment diffuses out into the culture medium. The sporulation begins on the sixth day (Table 4), and three zones develop in the conidial field (Fig. 2): greyish-yellow (1.6 cm) → light brown (0.3 cm) →bluish grey (0.4 cm).

3. On a Czapek agar culture medium containing Cu the orange-yellow pigmentation of the mycelium can be observed on the third day, and this diffuses out into the culture medium. The sporulation appears on the fifth day (Table 4). There is no zonation in the conidial field (Fig. 2), the entire colony being a homogeneous yellowish-grey.

4. On a Czapek-Dox agar culture medium containing Cu, Mn and Zn the pigmentation begins in the mycelium on the third day. The colony is orange-yellow, but the pigment diffusing out into the culture medium is light yellow.

5. On potato agar culture medium in white light the pigmentation in the mycelium begins only on the fifth day, and is organe-yellow in colour. The sporulation too is later, appearing only on the seventh day (Table 4). The centre of the colony is greyish-yellow (1.3 cm), followed by a 0.4 cm wide light brown ring, with finally a 0.3 cm grey zone (Fig. 2).

Table 4. Zonation, pigmentation and sporulation of *Penicillium notatum* on various culture media

Culture medium	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
Czapek (control)	5	3	5
Czapek + Mn	11	3	5
Czapek + Zn	3	4	6
Czapek + Cu	—	3	5
Czapek + Mn + Zn + Cu	11	3	5
Potato agar	3	5	7

Pigmentation and sporulation of *Aspergillus repens* on Czapek-Dox agar in various monochromatic lights

1. In white light the mycelium produces yellowish-green pigments, and a light green pigment diffuses out into the culture medium. Pigmentation can be observed on the fifth day, and sporulation on the seventh day (Table 5). The conidial field is a uniform green and there is no zonation (Fig. 1).

2. In blue light the pigmentation in the mycelium begins on the sixth day; this is yellowish-green and diffuses out into the culture medium. The sporulation begins only on the ninth day (Table 5). There is no zonation (Fig. 1), and the conidial field is a vivid green colour.

Table 5. Zonation, pigmentation and sporulation of *Aspergillus repens* in various lights

Light	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
White	—	5	7
Blue	—	6	9
Green	—	5	8
Orange	—	5	7
Red	—	5	7
Dark	—	4	6
Periodic	7	6	8

3. In green light the mycelium is yellowish-green, and the pigmentation begins on the fifth day. A light green pigment diffuses out into the culture medium. The sporulation can be observed beginning from the eighth day (Table 5), the conidial field is green, and there is no zonation (Fig. 1).

4. In orange-red light the colour of the mycelium is yellowish-green; the pigmentation begins on the fifth day (Table 5). A light green pigment diffuses out into the culture medium. The sporulation begins on the seventh day; there is no zonation (Fig. 1), and the conidial field is green.

5. In red light the mycelium is yellowish-green and a light green pigment diffuses out into the culture medium. The pigmentation of the mycelium begins on the fifth day, and the sporulation on the seventh day (Table 5). The spore mass is green; there is no zonation (Fig. 1).

6. In the dark the mycelium is yellowish-green, the pigmentation beginning on the fourth day (Table 5). Sporulation can be observed on the sixth day. The colour of the spores is dark green; there is no zonation (Fig. 1).

7. In periodic illumination a yellowish-green pigment is synthetized in the mycelium, and diffuses out into the culture medium. The pigmentation can be observed on the sixth day, and the sporulation on the eighth day (Table 5). Zonation occurs during the sporulation, and seven zones develop (Fig. 1): dark green (0.2 cm) → light green (0.3 cm) → yellowish-green (0.3 cm) → dark green (0.4 cm) → light green (0.2 cm) → yellowish green (0.2 cm) → dark green (0.2 cm).

Pigmentation and sporulation of *Aspergillus repens* in constant white light on culture media of various compositions

1. On Czapek agar culture medium containing Mn the mycelium is brownish-yellowish-green. The pigmentation begins on the fifth day. Pigment does not diffuse out of the mycelium into the nutrient medium. The sporulation can be observed from the seventh day (Table 4), and during the sporulation there is zonation (Fig. 2): brownish-green (0.3 cm) → yellowis-green (0.8 cm) → green (0.4 cm) brownish-green (0.4 cm), i.e. four different zones.

Table 6. Zonation, pigmentation and sporulation of *Aspergillus repens* on different culture media

Culture medium	Number of zones	Day on which phenomenon begins	
		Pigmentation	Sporulation
Czapek (control)	—	5	7
Czapek + Mn	4	5	7
Czapek + Zn	—	5	7
Czapek + Cu	—	5	7
Czapek + Mn + Zn + Cu	4	5	7
Potato agar	—	6	8

2. On Czapek-Dox agar culture medium containing Zn the colour of the mycelium is brownish-yellowish-green. The pigmentation begins on the fifth day, and an orange-yellowish-green pigment diffuses out into the culture medium. The sporulation can be observed from the seventh day (Table 6); it is greyish-green in colour, and there is no zonation (Fig. 3).

3. On Czapek agar culture medium containing Cu the mycelium in white light is brownish-green. The pigmentation begins on the fifth day. A brownish-

green pigment diffuses out into the culture medium, but it is of a lighter shade than that of the mycelium. The sporulation can be observed on the seventh day (Table 6); its colour is green, and no zonation can be distinguished (Fig. 3).

4. On Czapek agar culture medium containing Cu, Zn and Mn the pigmentation of the mycelium begins on the fifth day. A brownish-yellowish-green colour develops, which does not diffuse out into the culture medium. The sporulation can be observed from the seventh day (Table 6); zonation is weak, and the boundaries of the zones are indistinct (Fig. 2): brownish-green (0.3 cm) → yellowish green (0.9 cm) → green (0.4 cm) → brownish-green (0.5 cm).

5. On potato agar culture medium the colour of the mycelium is brownish-green. Synthetization of the pigments begins on the sixth day. A light yellowish-green pigment diffuses out into the culture medium. The sporulation appears only on the eighth day, and the spore mass has a brownish-green colour (Table 6).

Morphogenetic effects of the nitrogen sources

Since it was observed during our experiments that the presence of the different microelements in the culture medium frequently gave rise to essential colour modifications, the question arose of whether a similar role is also played by the macroelements forming the culture medium. By variation of the N-sources, an attempt was made to answer this question. Literature data were found that the fungi do not utilize nitrogen bound in different forms to the same extent (UBRIZSY and VÖRÖS, 1968), but no indication was given as to the effects of these on pigmentation and sporulation. Cultures were therefore grown on various culture medium combinations in which the $NaNO_3$ of the Czapek culture medium was replaced by $(NH_4)_2SO_4$, NH_4NO_3 and urea.

Our assumption was not completely groundless, for as can be seen from Table 7 the individual N-sources have different effects from the points of view of pigmentation and sporulation.

Table 7. Effects of various N-sources on the beginning of pigmentation and sporulation

N-source	Day on which phenomenon begins					
	Pigmentation			Sporulation		
	Asp. rep.	P. notat.	P. purpur.	Asp. rep.	P. notat.	P. purpun.
$NaNO_3$ control	6	5	6	8	6	8
$(NH_4)_2SO_4$	3	4	5	6	5	7
NH_4NO_3	4	4	5	6	5	7
Urea	12	11	10	13	13	13

Vitality of the spores taken from different zones, on the basis of the intensity of germination

Although neither the composition of the culture media nor the different types of light inhibited the sporulation completely, but merely delayed it, this does not mean that spores of the same vitality developed under all the study

conditions. In order to provide an answer to the above question, test germinations were carried out on every culture, and in a few special cases spores taken from zones of different colours and different ages at the same time were germinated and their growths followed.

In the first case the cover was removed from a sterile culture in a Petri dish, the culture was transferred to a sterile new culture medium, and the spores made to fall through by tapping the walls of the dish.

The results of the study confirmed that germinative spores developed on every culture medium, and in every variation of monochromatic light.

In the second study it was hoped to clarify which part of the colony is the one from which the germinative spores originate. The separate examination of the zones led to the following results:

1. Three rings developed on a colony of *Penicillium notatum* grown in the dark. The spores from all three rings germinated, but the rates of germination were different; the parameters involved were the time of appearance of the colony, and the rate of increase of the colony diameter (Table 8).

Table 8

Origin of the spore	Diameter (in cm) of the colony developing from the spore on the				
	1st	2nd	3rd	4th	5th day
zone 1	—	—	0.3	1.2	2.0
zone 2	—	0.2	0.6	1.6	2.4
zone 3	—	0.2	0.7	1.7	2.5

2. There is no zonation on a culture of *Penicillium purpurogenum* grown on a Czapek-Dox culture medium supplemented with Cu, but there are violet-brown spots in the brownish-grey conidial field. Spores were taken from both sites onto Czapek-Dox culture medium, with the results shown in Table 9).

Table 9

Origin of the spore	Diameter (in cm) of the colony developing from the spore on the				
	1st	2nd	3rd	4th	5th day
violet-brown	—	—	—	—	0.2
brownish-green	—	0.1	0.6	1.5	2.2

3. A culture of *Penicillium purpurogenum* on Czapek-Dox culture medium supplemented with Zn grows very weakly, but 5 zones develop during sporulation. The germinations of the spores originating from the different zones are given in Table 10.

4. The sporulation of *Aspergillus repens* exhibits zonation only in certain cases, for example on Czapek-Dox culture medium supplemented with Mn, where 4 zones develop. The germinations of the spores originating from the individual zones are shown in Table 11.

Table 10

Origin of the spore	Diameter (in cm) of the colony developing from the spore on the				
	1st	2nd	3rd	4th	5th day
zone 1	—	—	—	0.2	0.7
zone 2	—	—	0.2	0.6	1.2
zone 3	—	—	0.2	0.7	1.2
zone 4	—	—	—	—	0.2
zone 5	—	0.4	1.2	1.2	2.0

Table 11.

Origin of the spore	Diameter (in cm) of the colony developing from the spore on the				
	1st	2nd	3rd	4th	5th day
zone 1	—	—	0.2	0.6	1.2
zone 2	—	—	0.2	0.7	1.4
zone 3	—	—	0.4	0.9	1.6
zone 4	—	0.1	0.5	1.0	1.5

Microscopic study of the morphogetic changes

If the external factors are altered, not only the pigmentation and zonation but other changes too can occur in the morphogenesis; these latter are not visible with the naked eye, and accordingly the colonies were subjected to a thorough microscopic analysis which led to a number of positive results. Although significant differences were not found in the diameters of the conidiospores, the thicknesses of the hyphae, the metulae and the sterigmae, and in the sizes of the vesicles, the lengths of the conidiophores of *Aspergillus repens* varied in a characteristic way depending on the nature of the light. In the dark and in the long wavelength region, conidiophores generally 250 μ in length developed, whereas in white light and in the other monochromatic lights the length was 350–400 μ. For the other species examined, merely differences depending on the specific characteristics were observed, and the change of the external factors had no effect.

Discussion

The experiments led to the finding that the pigmentation and sporulation of the studied fungi are physiological processes which can be affected by the light and the nutrient. However, the effects do not appear uniformly for the individual species.

Photo-effects

The pigmentation and sporulation of the colonies depend on the nature of the light. Independently of the nature of the light, the pigmentation of the mycelium in *Penicillium notatum* is followed 2 days later by the sporula-

tion; in *Penicillium purpurogenum* and *Aspergillus repens* there is a 3–4 day interval between the pigmentation of the mycelium and the sporulation, which is the shortest in the dark, and the longest in blue light.

In many respects our results agree with the literature data. RAPER et al (1953) observed that the development of the reproductive organs in *Aspergillus orenatus* is inhibited by the light, but only blue light of short wavelength is effective.

The nature of the photoreceptors is still unknown. They were earlier considered to be carotinoids, and more recently as riboflavin (NARASIMHACHARI, 1963), and there is a fairly general conception that in the most light-sensitive fungi this same pigment acts as the photoreceptor of the sporulation (LUKENS, 1963).

The formation of the sporulation rings can also be affected by photoeffects. With the exception of periodic illumination, *Aspergillus repens* does not exhibit zonation, but here too the periodic variation of the temperature must be reckoned with as the inducing cause (Fig. 1). In the dark *Penicillium notatum* forms 3 rings, similarly as in monochromatic illumination, while the sporulation zonation is promoted by white light. The large number of rings formed in the periodic illumination can be attributed in part to the temperature changes. In *Penicillium purpurogenum* the zonation was enhanced by blue light, in contrast with the other two species, where the blue light was ineffective in this respect (Fig. 1).

The morphogenetic effect of blue light is marked in the development of the length of the *Aspergillus repens* conidiophore. While these are short in the dark or in red light, they attain a much greater length in blue light. Similar observations have been made by JONSON and HALPIN (1952). Photomorphogenesis was not observed in the other two species.

Culture medium effects

In addition to the light, the composition of the culture medium also plays a role in the generative development of the fungi.

Zn inhibits the pigmentation and the beginning of the sporulation in all three species. The shift in time amounted to 3–4 days. The most striking microelement effect was exhibited in the number of zones, in that the presence of Mn enhanced the zonation, whereas the presence of Cu decreased it. As regards the microelement effect, however, specific differences too were revealed, because *Aspergillus repens*, for example, in contrast to the other two species, forms sporulation rings only in the presence of Mn (Fig. 2). This phenomenon can be classified among the qualitative differences as opposed to the quantitative effects of the microelements, such as the fact that, on Czapek-Dox agar culture medium in the presence of Mn, *Penicillium notatum* forms twice as many zones as in the absence of Mn (Fig. 2). Similarly, in the case of Penicillium purpurogenum the addition of Mn enhances the formation of the rings, and in our experiments their number rose from 4 to 9. This effect of Mn requires further study, for the number of rings roughly doubled for the same colony diameter (Fig. 2).

The experiments in which the N-sources in the culture medium were varied led to the result that all three species utilize NH_4^+ most quickly, if the beginning of the colony formation is taken as the basis of the measure of utilization.

The second place is occupied by NO_3^-, and the third by the urea–N. The utilization of the three N-sources outlined above is confirmed by the beginning of pigmentation and sporulation too. Our findings are in contrast with those of UBRIZSY and VÖRÖS (1968), according to whom the N of NO_3^- is preferentially utilized by the fungi, although we agree with the finding that the utilization of NH_4^+ is a process requiring more energy than that for NO_3^-.

Literature data confirm that, even though to a restricted extent, the fungi utilize organic N–sources too (aminoacids, amides, peptides, proteins and nucleic acids (UBRIZSY and VÖRÖS, 1968).

We observed a new phenomenon in the relation of the zonation and sporulation: differences appear in the germination of the spores and in the intensity of their growth; the spores originating from the peripheral zones exhibit a greater vitality than the spores in the older zones.

References

BESSSEY, E. A. (1904): Über die Bedingungen der Farbbildung bei Fusarium. — Flora (Jena) *93*, 301—334.

CARLILE, M. J. (1965): The photobiology of Fungi. — Annual Review of Plant Physiology *16*, 175—199.

EBERHARD, D., W. RAU und C. ZEHENDER (1961): Über den Einfluss des Lichtes auf die Carotinoidbildung. — Planta *56*, 302—308.

FRIEDERICKSEN, J. und H. ENGEL (1960): Der Abschussrhythmus der Fruchtkörper von *Sphaerobulus stellatus*. — Planta *55*, 313—326.

JONSON, T. W. and J. E. HALPIN (1952): Influence of light on the morphology and production of conidia in some species of Dermatiaceae. — Phytopathology *42*, 342.

LUKENS, R. J. (1963): Photoinhibition of sporulation in *Alternaria solani*. — Amer. J. Bot. *50*, 720—724.

MOHR, H. (1961): Wirkungen kurzwelligen Lichtes. In Handbuch der Pflanzenphysiologie XVI. 439—531, Ruhland, W., Ed., Springer, Berlin.

MUNTANJOLA—CVETKOVIC, M. and M. NESKOVIC (1968): Studies on the effect of some substances as possible substitutes of light in the sporulation of *Aspergillus flavus* 28—A. — Bull. de l'Institut et du Jardin Botaniques de l'Université de Beograd.

MUNTANJOLA. CVETKOVIC, M., M. NESKOVIC and L. J. CULAFIC (1968): Morphogenetic responses of *Aspergillus aureolatus* Munt.—Cvet. — Bull. de l'Institut et du Jardin Botaniques de l'Université de Beograd.

NARASIMHACHARI, (1963): Studies on the pigments of *Penicillium berquei*. — Canad. J. Chem. *41*, 641—648.

RAPER, K. B., D. J. FENNEL and H. D. FRENSER (1953): The ascosporic stage of *Aspergillus citrisporus* and related forms. — Mycologica *45*, 671.

SÁRKÁNY, S. és SZALAI, I. (1966): Növényszerkezettani gyakorlatok, Tankönyvkiadó, Budapest.

SZALAI, I. és FRENYÓ V. (1962): Növényélettani kísérletek. Tankönyvkiadó, Budapest.

TATARENKO, E. S. (1954): Der Einfluss von Lichte auf die Entwicklung von Schimmelpilzen. — Microbiologia *23*, 29—33.

UBRIZSY, G. és VÖRÖS J. (1968): Mezőgazdasági mycologia. Akadémiai Kiadó, Budapest.

WITSCH, H. V. und F. WAGNER (1965): Beobachtungen über den Einfluss des Lichtes auf Mycel- und Konidienbildung bei Alternaria brassicae Var. dauci. — Arch. Microbiol. *22*, 307—312.

ZALOKAR, M. (1954): Arch. Biochem. Biophys. *50*, 71—80.

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DORMANCY IN FRUITS OF TILIA PLATYPHYLLOS SCOP. I

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Summary

A study was made of the dormancy of seeds of *Tilia platyphyllos* SCOP., with special regard to the clarification of the roles of the pericarp, the testa and the endosperm in the maintenance of the seed dormancy. It was found that under moist conditions the seeds require several months at low temperature (5–6 °C) to pass through the after-ripening, which is the condition of germination. The seeds have no need for a photoperiod, light and darkness both being ineffective. Germination is hindered by the presence of the thick pericarp and the seed-coat, which prevent the washing-out of the inhibitors. The inhibitors cannot be removed from the intact seeds by washing, whereas they can be washed out of the bare seeds (without seed-coat) in 24 hours. The excised embryos grow on moist filter paper and on *White* culture medium with a constant intensity, independently of the pre-treatment. The germination is not stimulated by thiourea, KNO_3 or ethylene chlorohydrin, and even the effect of gibberellic acid is very moderate.

Introduction

Tilia platyphyllos SCOP. is widespread throughout the whole of Europe; it mainly forms woods in mountainous regions, but it is also planted. The ssp. pilous on the leaf veins was selected for study. Its unopening, unilocular fruit normally contains one seed (Fig. 1). The fruits are in deep dormancy, and under normal conditions their germination occurs in the second spring following the formation of the fruit. In forestry practice the fruit is stratified into sand in the open (kept at 3–5 °C for about 150–180 days), and then sown in the second spring.

The freshly gathered seeds of a considerable proportion of the woodland trees indigenous to the temperate zone are in dormancy and require an after-ripening period at low temperature (5 °C) to become capable of germination. Attempts have been made to elucidate the basis of the mechanism of dormancy by the detailed study of the biochemical processes occurring during the after-ripening (COLMAN, 1961; FRANKLAND, 1961; PINFIELD, 1965; FRANKLAND and WAREING, 1966), but those changes which can be followed during the after-ripening, such as the increase in activity of the enzymes and the mobilization of the reserve substances, can also be observed on the germination of

the seeds without dormancy, and it thus appears that they are not closely connected with the after-ripening.

It seems more significant to study the relation of growth-stimulating hormones and inhibitors in the extracts by chromatography, coupled with appropriate biotests. For example, the decrease of the amount of inhibtors in *Acer* (PHILLIPS and WAREING, 1958) is in correlation with the cessation of the bud dormancy. The inhibitors have a confirmed role in the seed dormancy in *Xanthium* (WAREING and FODA, 1957), *Betula* (BLACK and WAREING, 1959) and *Fraxinus* seeds (VILLIERS and WAREING, 1960; SZALAI, 1965, SZALAI and NAGY, 1968).

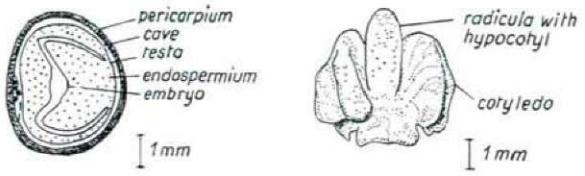

Fig. 1. Cross-section of fruit of *Tilia platyphyllos* Scop. (A) and embryo excised from the endosperm (B).

In contrast to the fairly abundant literature data referring to the dormancy of *Fraxinus, Acer, Corylus* and *Fagus* sp. seeds, no literature data were found for *Tilia platyphyllos* as regards the state of dormancy of the fruits or seeds.

An account is given below of our experimental results, the aim of which was to establish:

1. how fruits and seeds imbibed with water behave under normal germination conditions in the period following the ripening of the fruit;
2. whether there is a difference in the behaviour of fruits and seeds subjected to low temperature;
3. whether the embryo begins to grow in the imbided seeds;
4. the effect of washing out the fruits and seeds with water;
5. the germination-stimulating effects of various dormancy-terminating agents.

Materials and Methods

The study material was obtained from the Csongrád County Forestry Station; it originated from trees forming a closed plot, and thus population material was studied. Petri dishes, washed sand and Schleicher—Schüll filter paper were used for the germination tests, with boiled water for the moistening. The fruits and seeds were preliminarily sterilized with 0.1% sublimate solution.

Variations:

1. Germination:
   a. in constant light (6000 lux, at 6 °C and 25 °C);
   b. in constant darkness (at 6 °C and 24 °C);
   c. in 12-hour alternating periods of light and darkness (at 24 °C).
2. Seeds germinated under the conditions given in (1) were prepared at 25-day intervals, and the development of the embryo was examined.
3. The stratification was performed in a refrigerator at 4—5 °C.
4. The fruits were soaked in distilled water and mildly shaken for 48 hours, and then subjected to germination as under (1).
5. After scarification the fruits were treated with thiourea, ethylene chlorohydrin, potassium nitrate and gibberellin.

Results

1. Germination tests

Immediately after the falling of the fruit, the fruits and seeds were sown in washed sand in Petri dishes, but to facilitate the imbibing they were treated with concentrated sulphuric acid. Since sulphuric acid treatment involves the danger of the acid penetrating the hilum and destroying the tissues of the seed, it was considered advisable to investigate the connection between the various times of scarification and the extent of damage. On this basis a „pickling" time of 15 minutes appeared to be the most effective. The relation between the time of sulphuric acid treatment and the swelling is given in Fig. 2.

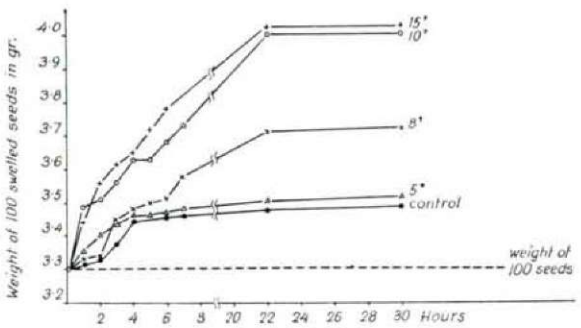

Fig. 2. Relation of the duration of the sulphuric acid scarification and the time necessary for the swelling of the seeds.

Efforts to induce germination immediately following the falling of the fruit did not lead to success, for there was practically no germination during 120 days in constant light (at 25 °C), in constant darkness (at 24 °C), or in alterating light and darkness (at 24 °C); only a few seeds burst open (7% maximum), but the radicle did not emerge. Under similar conditions but at 6 °C bursting of the seeds occurred in a higher percentage (> 50%), but even then the radicle did not appear.

2. Development of the embryos in the swollen seeds

When the seeds were opened it was found that under the most varied germination conditions (1a, 1b, 1c), both in the cold and at laboratory temperature, the embryos remained in dormancy (Fig. 3). On the other hand, if the excised embryos were placed into *White* nutritive solution or moist filter paper in Petri dishes, they began to grown. From a graph showing the growth on the *White* medium (Fig. 4) it can be seen that the embryos grew here with an approximately constant intensity, regardless of the nature of the pretreatment; this indicates that the growth of the embryo is inhibited by the endosperm or the seed-coat, or by their joint presence.

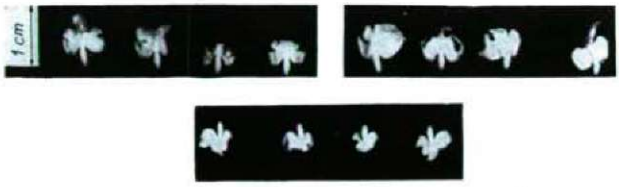

Fig. 3. The development of embryos excised from *Tilia* seeds after various pre-treatments. From left to right in pairs: in constant light at 6 °C and 24 °C, in constant darkness at 6 °C and 24 °C, and in 12-hour light and dark periods at 24 °C.

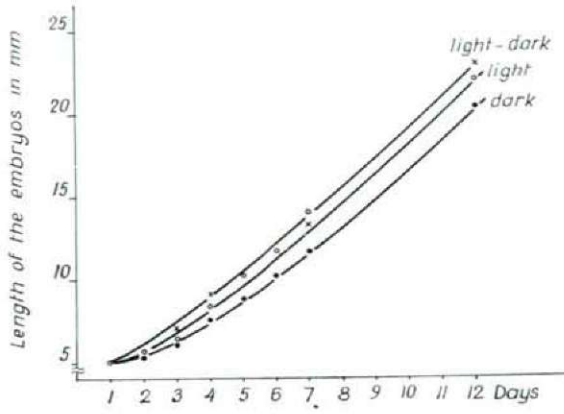

Fig. 4. Development on *White* medium of excised *Tilia* embryos after various pre-treatments, on the 1st—12th days following the excision.

3. Stratification

For many species the imbided seeds can be aroused from their state of dormancy by subjection to low temperature. The stratification of the *Tilia* seeds paper wadding, and in the other case in moist sand for 3 months at 6 °C, and the germination was then followed at 20 °C. It was found that 65% of the seeds stratified in the sand germinated in the fourth month of the treatment, whereas only 60% of those in the paper wadding germinated and only in the fifth-sixth months. The proportion and vigour of germination are shown in Fig. 5.

4. Effect of washing out seeds and the embryo

It was assumed that the inhibitors maintaining the dormancy of the seeds are water-soluble, and accordingly can be removed from the seeds by washing. The scarified seeds were washed for 24 hours under running water, and then for a further 24 hours in water changed every 2 hours, with gentle shaking. This was followed by germination according to (1).

5. Effects of various dormancy-breaking methods

The fruits were scarified, then treated with thiourea, ethylene chlorohydrin, KNO_3 or gibberellin to stimulate the germination, and finally placed in a moist environment. All these substances have been employed with good results to terminate the dormancy in various species. With the exception of gibberellin, none of the techniques used led to germination in the present case; the cause of this considered to be that the substances did not penetrate into the seeds to a sufficient extent. Only in the case of gibberellin was a slight stimulating effect observed, when the gibberellin treatment was followed by germination at low temperature. Gibberellin was used to stimulate the scarified seeds in various concentrations, with the results shown in the Table.

It can be concluded from the results that GA_3 in itself can not be regarded as the initiating factor, for its effect, which was moderate, appeared only in the chilled seeds.

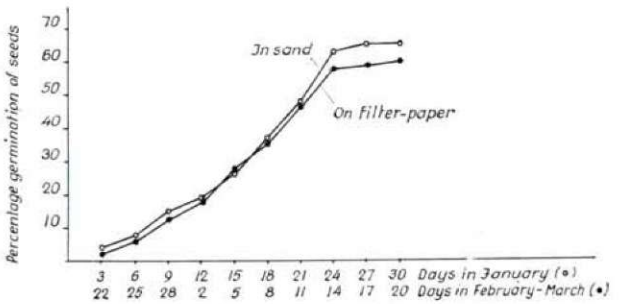

Fig. 5. Germination of seeds stratified in moist sand and in moist filter paper at 20 °C.

Discussion

It was demonstrated by WAREING and FODA (1957) that the inhibitors can be removed from excised *Xanthium* embryos by washing, and such ebryos germinate on a moist substrate. The washing-out was not successful in the case of the intact *Tilia* seeds, whereas according to STEINBAUER (1937) and FERENCZY (1955) the inhibitors can be removed from *Fraxinus* fruits even in the presence of the seed-coat. As regards practice, only this latter case would be of importance. Since the dormancy of the intact *Tilia* seeds can not be terminated by washing-out, it must be assumed that the washing-out of the inhibitors is hindered by the seed-coat, or the inhibitor is not water-soluble. In the latter case the destruction of the inhibitors under natural conditions leads to germination. Since the scarified seeds swell relatively quickly, the water-permeable seed-coat freely permits the passage of oxygen too.

It is worthy of note that the various stimulating agents (ethylene chlorohydrin, KNO_3, thiourea) have no effect, a slight stimulation being induced only by GA_3 treatment. These facts indicate that the primary factor in the dor-

Illumination	Constant light 6000 lux						Constant darkness						Alternating light and darkness						Constant darkness					
Temperature	25 °C						25 °C						25 °C						5 °C					
GA_3 concn.	1000	500	200	100	50	control	1000	500	200	100	50	control	1000	500	200	100	50	control	1000	500	200	100	50	control
1. II., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10. II., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20. II., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1. III., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10. III., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1	3	2	—
20. III., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	7	7	4	4	—
1. IV., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	6	9	11	12	5

mancy of the *Tilia* seeds is the presence of the inhibitors; their remaining in the seed is enhanced by the thick pericarp and the impermeable seed-coat. The first step towards the understanding of the nature of the dormancy must be the analysis of the inhibitor contents of the individual seed-parts.

References

BLACK, N. and P. F. WAREING (1959): The role of germination inhibitors and oxygen in the dormancy of the light-sensitive seed of *Betula* species. — J. Exp. Bot. *10*, 134.

COLMAN, B. (1961): Metabolic aspects of dormancy. — Ph. D. Thesis, Univ. of Wales.

FERENCZY, L. (1955): The dormancy and germination of seeds of *Fraxinus excelsior*. L. — Acta Biol. Szeged, *12*, 17.

FRANKLAND, B. (1961): Effect of gibberellic acid, kinetin and other substances on seed dormancy. — Nature, London *192*, 678.

FRANKLAND, B. and P. F. WAREING (1966): Hormonal regulation of seed dormancy in hazel and beech. — J. Exp. Bot. *17*, 596—611.

PHILLIPS, I. D. J. and P. F. WAREING (1958): Studies on dormancy of Sycamore. I. Seasonal changes in the growth substance contant of the shoot. — J. Exp. Bot. *9*, 350.

PINFIELD, N. (1965): Gibberellic acid and seed dormancy. Ph. D. Thesis, Univ. of Wales.

STEINBAUER, G. P. (1937): Dormancy and germination of *Fraxinus* seeds. — Plant Physiol. *12*, 813.

SZALAI, I. (1965): Brechung der Keimruhe von *Fraxinus* Samen durch Gibberellinsäure. — Acta Biol. Szeged *11*, 93—100.

SZALAI, I. and MÁRIA NAGY (1968): The causes of dormancy in *Fraxinus excelsior* L. fruits and the role of gibberellic acid in breaking dormancy. III. The stimulative and inhibiting susbtances of the fruits. — Acta Bot. Acad. Sci. Hung. *14*, 415—423.

VILLIERS, T. A. and P. F. WAREING (1960): Interaction of growth inhibitor and a natural germination stimulator in the dormancy of *Fraxinus excelsior*. — Nature, London *185*, 112.

WAREING, P. F. and H. A. FODA (1957): Growth inhibitors and dormancy in *Xanthium* seed. — Physiol. Plant. *10*, 266.

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EFFECTS OF THE NUTRIENT COMPOSITION AND THE NATURE OF THE LIGHT ON THE GROWTH AND PIGMENT-SYNTHESIS OF SOME MOULDS

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Summary

Investigations were carried out with the species *Penicillium notatum, Penicillium purpurogenum, Aspergillus repens* and *Aspergillus versicolor*. Their growth intensities and pigment-syntheses were studied as functions of the culture medium and the nature of the light. The findings were as follows:

1. The composition of the culture medium and the nature of the light each affect both the development and pigmentation of the fungi.

2. It can be said in general of the species included in the experiments that the growth of the colonies is completed in the 8th week.

3. Microelements added to the culture medium have different effects on the individual species, in some cases stimulatory, in others inhibitory. The growth is strongly inhibited by Zn, but slightly stimulated by Mn, and more strongly stimulated by Cu.

4. Comparison of the data show that *Penicillium notatum* is not light-sensitive, while the growth of *Penicillium purpurogenum* and *Aspergillus repens* is inhibited to a lesser extent by green and orange light, and to a greater extent by blue light; the growth is enhanced compared to that in white light by red light and darkness; an exception to this, however, is the growth in periodic illumination.

5. The light and culture medium also exert effects on the pigmentation.

6. The quantity and quality of the pigment content increase in every species during development.

7. The pigment composition of the *Aspergillus* species is more variable than that of the *Penicillium* species.

8. It is a generally valid tendency that the pigment content in orange and green illumination and in the dark is poorer than in white and red light.

9. The absorption curves of the pigments of colonies grown on various culture media show that the pigments synthetized by the fungi are mixtures of several components. No matter what the variations in the pigments, their absorption maxima lie in the range 400–550 nm. There is no absorption in the yellow, the red or the infrared.

Introduction

The important role of the pigments of various colours in the lives of plants is well known. Vital photosynthesis can occur only with the chlorophyll pigments. The presence of phytochromes is decisive in the development of many phenomena of life, such as photoperiodism, phototropism and the various photomorphoses. The carotinoids and flavins play a role as photoreceptors in the various movements. Finally, we can mention the biological importance of flavonoids, providing flowers with colour.

It is also well known that the various fungi are coloured by a whole series of pigments, but far less is understood about the varied forms of the pigments and their importance than for the higher-order plants, despite the large number of papers published on this topic.

Strains were selected for the experiments from the *Aspergillus* and *Penicillium* genera, partly on the basis of their rapid and simple cultivation, and partly because of the varied colours of their pigments. Accordingly, 10 *Penicillium* and 20 *Aspergillus* strains were selected, the most suitable of which proved to be the species

Penicillium purpurogenum	(787)*
Penicillium notatum	(190)
Aspergillus repens	(–)
Aspergillus versicolor	(710)

A decisive question with regard to the selection was the separability of the pigments. It was observed during the preliminary experiments that the pigments could not be successfully separated from their carriers with the organic solvents generally and widely used for pigment extracts. On the other hand, though it did prove possible to separate them by means of protein denaturing procedures (e.g. acidic hydrolyses), at the same time the pigments underwent changes in colour.

In accordance with our working hypothesis, a closer study was made of:

a. the effects exerted on the growth and the pigment-synthesis by culture media differing fundamentally from each other in composition;

b. the qualitative effects exerted by various monochromatic lights on the growth and pigment-synthesis in the case of strains cultivated on a given culture medium;

c. the intensity of pigment-synthesis and the quantitative relations in both cases, i.e. as functions of the culture medium and the light.

With the data obtained in our studies we wished to make a contribution to the elucidation of a region of plant physiology which has not yet been sufficiently investigated; it was assumed that the pigments are not useless metabolic products in the fungi, but metabolic products which are affected by the composition of the culture medium and the nature of the light, and which may play a role in the vegetative and reproductive development by means of their various absorption properties.

* The numbers in brackets denote the strain cultures maintained in the Viticultural and Oenological Research Institute.

The justification of our assumption is supported by a number of literature data. According to studies with microspores (CARLIE, 1965; MOHR, 1961), the light exerts significant growth-stimulating and growth-inhibiting effects alike. Since only the light absorbed by the cell is effective, it follows that the pigments may play a decisive role in both the growth and the multiplication of the fungi. Literature data can also be found which indicate that the light effect depends too on the composition of the culture medium (CARLILE, 1965; MUNTANJOLA et al., 1968). When cultivated on malt agar, *Penicillium clavigerum* is insensitive to light and grows with the same intensity in dark and light, whereas on CZAPEK's solution agar its rate of growth decreases on a 24-hour illumination, but remains constant on a daily 12-hour illumination. *Penicillium claviforme* requires light only at the beginning of development (CARLILE, 1965), until the mycelia have attained a length of some millimetres, and after this are apparently insensitive to illumination. In contrast, *Penicillium isariiforme* is sensitive to light throughout its entire life. What has been said would suggest that even the species belonging to a given genus react completely differently to light, depending at times on their developmental state, and at other times on the composition of the culture medium. On the other hand, this indicates that the sensitivity or indifference of the species to light is not connected with the kinship.

The synthesis of the pigments is explained by the „metabolism" theory in that the formation of certain metabolic products requires light and does not take place in the dark (CARLILE, 1965). It was observed on fungi grown in light and in dark that the development of their colours, and their varieties of colour, depended on the light.

It is a generally accepted view that light is necessary, primarily for carotenoid production. It has been found that *Fusarium aqueductum* (EBERHARD et al., 1961) and *Pyronema confluens* (CRALILE, 1956) synthetize carotenoid only in light, whereas light merely enhances the carotenoid synthesis in *Phycomyces blakesleeanus* (GARTON et al., 1951).

The light not only exerts an effect on the carotenoid-type pigments, but, for example, also inhibits the formation of naphthoquinone in *Fusarium osysporum*.

Melanin formation is affected by light in contrasting ways: it is stimulated in *Aureobasidium pullulans* (LINGAPPA, 1963), and inhibited in the black mutant of *Neuspora crassa* (RAPER, 1949). It is of great interest that pigments formed in the dark are reversibly transformed to other colours as a result of photo-oxidation if the fungus is illuminated. Several examples of this were found in our own experiments, in agreement with the data of RIEDHART and PORTER (1958), who observed the transformation of the yellow pigment in *Penicillium herguei* to a green pigment.

Also of interest are the results of a study of whether the photo-effect can be substituted, and if yes, to what extent with the change of the composition of the culture medium (MUNTANJOLA et al., 1968).

These observations show that none of the sugars is able to substitute the effect of light, but in high concentration a weak sporulation can be induced. In light, however, the composition of the culture medium causes changes in the pigmentation.

Besides the generalities, the literature does not provide sufficient information with regard to the effects of various monochromatic lights and various substrates on pigment-synthesis.

Materials and Methods

1. Experimental objects: *Penicillium purpurogenum, Penicillium notatum, Aspergillus repens* and *Aspergillus versicolor* strains. The strains were provided by the National Viticultural and Oenological Research Intstitute; for the duration of the experimental period they were kept on a potato glucose agar medium in test-tubes, under a protecting layer of paraffin oil, and these accurately determined strains were used for the transoculations.

2. Culture media: The following five culture media were used to study the effects of the medium:

a. Czapek-Dox solid culture medium (pH 6.8—6.9) to which the sugar was added before the sugar was added before the final sterilization.

b. Czapek-Dox culture medium supplemented with microelements in the following variations:

1.	Basal nutrient medium (a) + $CuSO_4 \cdot 5\,H_2O$	0.0025	g
2.	Basal nutrient medium (a) + $MnSO_4$	0.0025	g
3.	Basal nutrient medium (a) + $ZnSO_4$	0.0025	g
4.	Basal nutrient medium (a) + $MnSO_4$	0.001	g
	+ $ZnSO_4$	0.001	g
	+ $CuSO_4 \cdot 5\,H_2O$	0.001	g

c. Potato agar culture medium (Szalai and Frenyó, 1962).

d. Czapek-Dox liquid culture medium.

e. Modified Czapek-Dox culture medium, the basal composition of which agreed with that of culture medium (a), but the C cource was varied and it was supplemented with vitamin B in the following variations:

(1) Varying the C source, 10% or 20% dextrose was employed in place of 30 g (3%) dextrose.

(2) Culture medium (c) was supplemented with a yeast extract.

The culture media were freshly prepared in all cases and, after adjustment to the appropriate pH, were poured according to the aims of the experiment into test-tubes 1.5 cm in diameter, Petri dishes 10 cm in diameter, or 150 ml Erlenmeyer flasks.

After sterilization the culture media were put into room-temperature thermostats for 3—4 days, and only those were used for inoculation which proved to be sterile. The spore or mycelium pieces were transferred onto the appropriate sterile culture medium in a UV-sterilized chamber by means of an inoculating loop, in such a way that it adhered well to the surface of the agar, but did not sink in deeply and into the culture medium.

Under the (usual) sterile conditions, infection-free cultures were atteined in 80% of the transoculations.

The solid culture media were used to study the intensity of growth and the structure of colony formation, while pigments were extracted from the cultures of the liquid media.

In both cases 30 ml of nutrient solution was employed.

3. Cultural procedure (incubation)

The cultural conditions were varied according to the aims of the experiment. The cultures were placed in thermostats, kept in the dark, and developed at 26—28 °C (± 0.2 °C). The cultures subjected to variation by night and day were maintained at laboratory temperature of 22—33 °C.

For the study of the photo-effect the cultures were placed in a climatic chamber, the temperature of which was similarly 26—28 °C (± 1 °C). The climatic chamber was illuminated with light of an intensity of 1200—1500 lux, enriched with monochromatic light by the use of white, red, orange, green and blue discharge tubes.

The growth of the cultures was expressed with the index of transverse diameters, and the measurements were made at gradually increasing intervals (on the 1st, 2nd, 3rd, 4th, 6th, 8th, 10th and 12th days).

4. Pigment extraction

The cultures on the liquid culture medium were separated on a Büchner funnel on the intense pigmentation of the culture medium (generally after 14—16 days), the mycelium mass homogenized, and the pigments were dissolved out by treatment with methanol. Two methods were used during the procedure. In one case the culture was subjected to a fractionation procedure, an aqueous extract (fraction II) was prepared from the mycelium mass after homogenization, and the residual pigments were eluted with methanol during 24 hours (fraction III). The nutrient solution separated from the mycelium mass (fraction I) was subjected to spectrophotometric analysis after evaporation, similarly to the other two fractions.

In the other case the methanolic extract of the mycelium was combined with the filtrate, and used for chromatography after methanolic extraction and mild evaporation.

5. Chromatography of the pigments

Thin layers were prepared from silica gel according to the method of STAHL (1967) and applied in a thickness of 1 mm to 20×20 cm glass plates. After drying for 10 minutes the plates were activated at 50 °C. 0.1 ml portions of the material were transferred to the start-point by micropipette and a chloroform : acetic acid mixture (7 : 2 v/v) was used as solvent; this had been found to be the most suitable after trials with a large number of running solvents. The development was carried out at room temperature, a time of 80—90 minutes being required for the attainment of a front distance of 15 cm.

The fractionated pigment components were also studied in UV light.

Results and discussion

Dependence of growth on the composition of the culture medium

For every culture medium it can be said that the colonies of the species selected for the experiment grew quickly and the pigmentation, zonation and colony character typical of the species had developed on the 6th day following the transoculation. After the 6th day the inner parts of the colony multiply in thickness, but in contrast the peripheral part remains thinner and the hyphal mat is looser. The colony becomes increasingly thick on ageing.

It is also generally valid for the cases studied that the growth of the

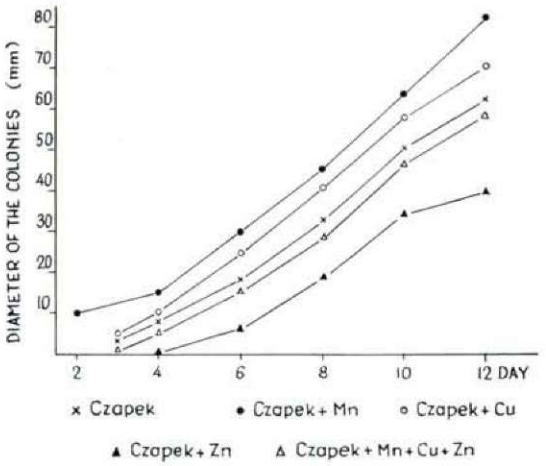

Fig. 1. Growth of *Penicillium notatum* in white light on Czapek-Dox agar culture medium enriched with microelements.

colony is completed in the 8th–10th week, the mycelia harden, and their water content decreases.

The growths of *Penicillium notatum* and *Pencillium purpurogenum* on Czapek-Dox agar were measured in white light and the obtained results were used as controls for the growths on culture media of different compositions (Figs. 1, 2).

Of the microelements, Zn inhibited the growth, while Mn stimulated it to a greater extent and Cu to a lesser extent compared to the basal media. The use of the three microelements together did not result in as large a growth as for Mn or Cu alone; thus, the inhibiting effect of Zn was exhibited even in the combination of the microelements (Fig. 1).

The growth of *Penicillium purpurogenum* was similar to that of *Penicillium notatum* on the basal medium. The inhibiting effect of Zn is striking, particularly at the beginning of the development, and in this case the stimulating effect of Cu exceeds that of Mn. It is worthy of note that the combined application of the three microelements stimulates the growth in the first ten days of the development better than the microelements separately, and the inhibiting effect of Zn appears only in the later stage of the development (Fig. 2).

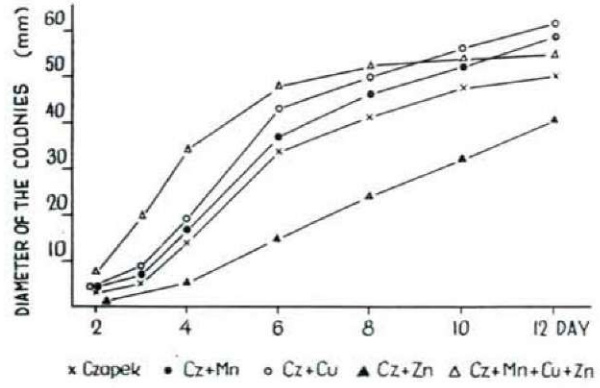

Fig. 2. Growth of *Penicillium purpurogenum* in white light on Czapek-Dox agar culture medium enriched with microelements.

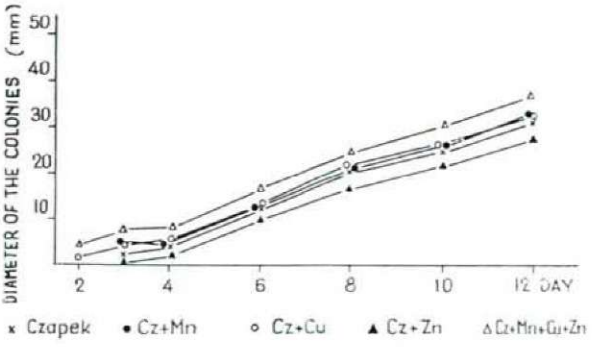

Fig. 3. Growth of *Aspergillus repens* in white light on Czapek-Dox agar culture medium enriched with microelements.

The composition of the culture medium has only an extremely small effect on the growth of *Aspergillus repens*. The curves shown in Figure 3 are practically parallel to each other, and the differences between them are so small that they may be considered to be within the limits of error. With the exception of Zn, the microelements exert a just measurable stimulation, while Zn exhibits an extremely slight inhibiting effect.

As regards the given culture media, therefore, *Aspergillus repens* is not sensitive.

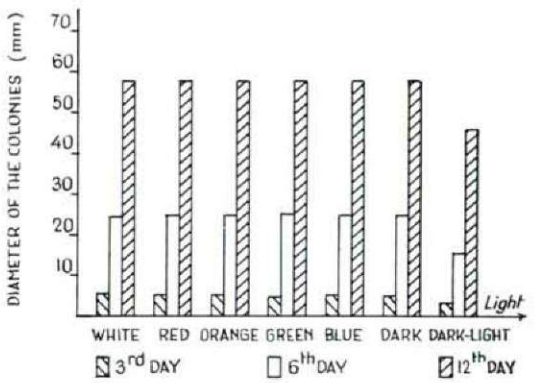

Fig. 4. Growth of *Penicillium notatum* on Czapek-Dox agar culture medium in various monochromatic lights.

Photo-effects on the growth on Czapek-Dox agar culture medium

Significant differences in growth were observed in the various spectral regions enriched with monochromatic light as detailed in the methodological description (Figs. 4–6). For the better illustration of the differences column graphs were used, and to show the differences more distinctly only the experimental data for the 3rd, 6th and 12th days are given. Growth in white light was again taken as control, and the growths observed in the monochromatic light was compared to this.

In the case of *Penicillium notatum* the monochromatic light induced no stimulation or inhibition at all, but in periodic illumination the growth decreased, and thus *Penicillium notatum* can be considered a photophilic fungus. This photophilia, however, can be satisfied not only by white light but by any monochromatic light. The 1-mm differences occurring here and there in the colony diameter are probably due to errors of measurement (Fig. 4).

The photo-effects are just the reverse in the case of *Penicillium purpurogenum*. If the growth observed in white light is used as control, then the growth in the dark can be said to be more intense than in light. The strong decrease of the growth measured in periodic illumination can not be explained by the scotophilia. Of the monochromatic light regions, red light has almost the same effect as white light, while orange, green and blue lights exhibit

increasing inhibiting effects on the growth. It can be concluded from the experimental data that the growth of *Penicillium purpurogenum* is inhibited to an increasing extent with the shortening of the wavelength of the light (Fig. 5).

Aspergillus repens behaves similarly to *Penicillium purpurogenum* and grows much more quickly in the dark than in constant white light. The growth is also greater in red light than in white light, but decreases in all the other spectral regions in proportion to the shortening of the wavelength. In periodic

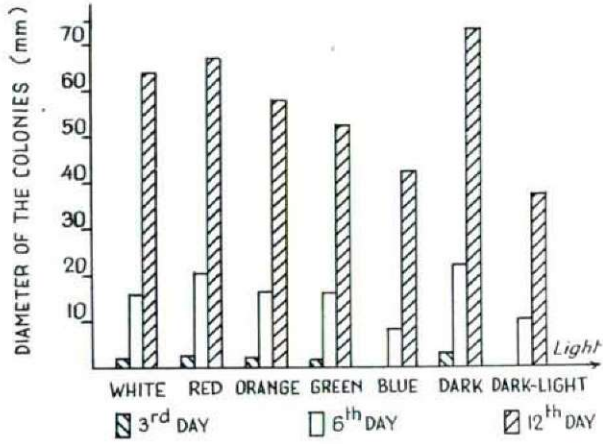

Fig. 5. Growth of *Penicillium purpurogenum* on Czapek-Dox agar culture medium in various monochromatic lights.

illumination the growth-stimulating effect of the dark period is more weakly manifested than the inhibiting effect of the light (Fig. 6).

It can be established from the above that for the three moulds examined

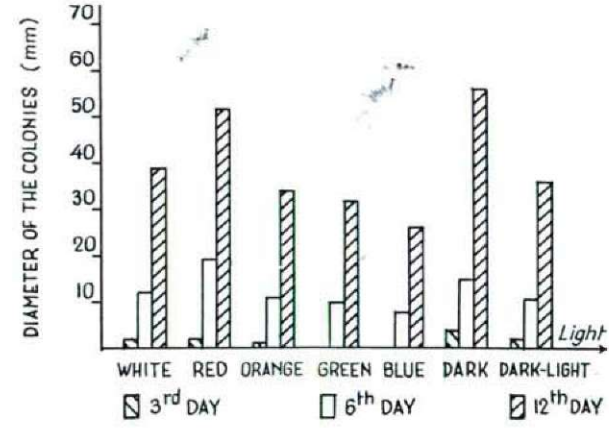

Fig. 6. Growth of *Aspergillus repens* on Czapek-Dox agar culture medium in various monochromatic lights.

the nature of the light plays a larger role in the regulation of the rate of growth than the composition of the culture media studied.

The next question is whether this effect of the two factors occurs in the synthesis of the pigments.

Photo-effects and variation of the pigment content

Unexpected difficulties were encountered in the extraction and separation of the pigments, and this compelled us to simplify the planned complex investigations. The difficulties arose from the fact that the chromatographic separation of the components succeeded only after long experimentation, as already indicated in the methodological part. Even with extraction and running in decreased light, the individual colour components, although always identifiable, appeared with very faint colours, and in ordinary light the contours of the spots could not be identified in most cases. It was effective in almost every case to study the thin layers in UV light (254 m), when the colours were intensified and the contours too became distinguishable. The following can be said in connection with the pigment variation:

In *Penicillium notatum* a maximum of 7 spots could be distinguished in the red on the 14th day. Pigments of colonies grown in orange illumination gave 5 spots. The pigments synthetized in the different illuminations are given in Figure 7, and we should like to indicate the differentiation og these. The chromatogram confirms that the colour composition of *Penicillium notatum* varies in quality primarily depending on the illumination.

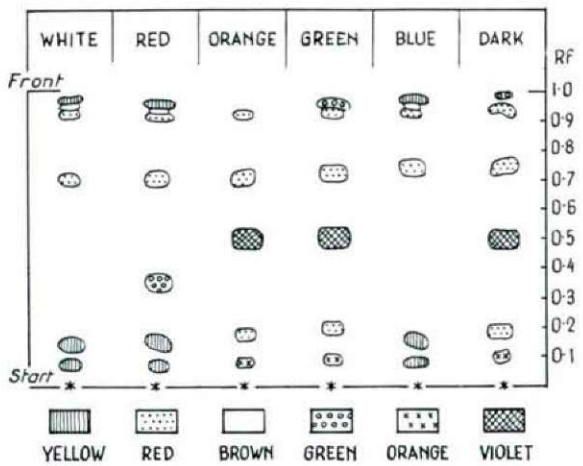

Fig. 7. 14-day *Penicillium notatum* pigments separated on a silica gel thin layer.

The number of components increases in the pigments extracted on the 24th day; this is most striking in colonies developing in the dark, where 10 components can be recognized, and becomes predominant in red, brown and bluish-violet colours. It can be stated in connection with *Penicillium notatum*

that in the older colonies the green and *bluish*-violet components accumulate (Fig. 8), these not being characteristic for young colonies (Fig. 7).

The richness of colour of *Penicillium purpurogenum* is greater than that of *Penicillium notatum.* A maximum of 9 and a minimum of 6 colour-spots were distinguished on the 14th day. The most spots occurred in blue and white lights. The fewest spots were given by the chromatogram of an extract of fungus grown in the dark. In contrast with *Penicillium notatum,* the green spot here with an Rf value of 0.37 appears for all of the monochromatic lights.

In *Penicillium purpurogenum* the red colour exhibits three different Rf values, and is present in such large quantity that the culture medium is coloured a completely homogeneous red. In the dark, or in orange and green lights a green pigment appears at Rf 0.12; this is not present in red light.

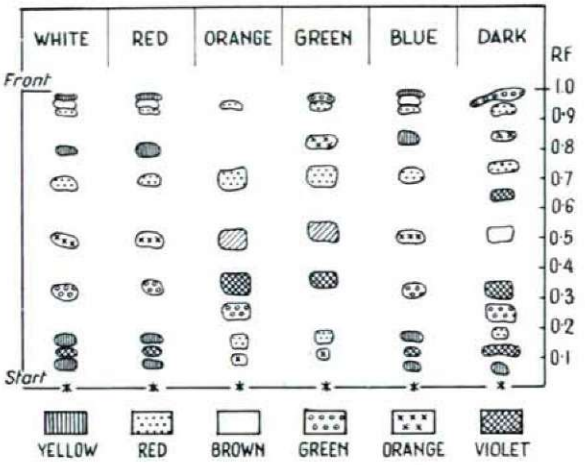

Fig. 8. 24-day *Penicillium notatum* pigments separated on a silica gel thin layer.

The chromatogram of pigments extracted on the 24th day is even richer in colour, and the number of components is higher. At most 12 components appear; this in due in part to new red spots and shades of green. In the dark, however, the pigment content remained unchanged even on the 24th day.

In *Aspergillus repens* a minimum of 4 and a maximum of 8 components appear on the 14th day: the fewest in orange or darkness, the most in red light.

On the 24th day 13 pigments are found in the cultures in blue light, and only 8 pigments in those in the dark. The compositions of the pigments exhibit many similarities in white, red and blue lights, and in orange and green lights.

The pigment content of *Aspergillus versicolor* is the most varied, 14 pigments appearing in the 14-day culture.

The most varied pigment content is synthetized in white light, and the least (5 spots) in the dark. In this respect, therefore, the two *Aspergillus* are similar to each other. The variety of the pigments increases with the growth

of the culture; the red, and then the yellow and yellowish-green pigments dominate, while the blue pigments have the lowest contents (Fig. 9). The young colonies are richer in colour in red light than in blue light, but the situation is the reverse in the older colonies (Fig. 10).

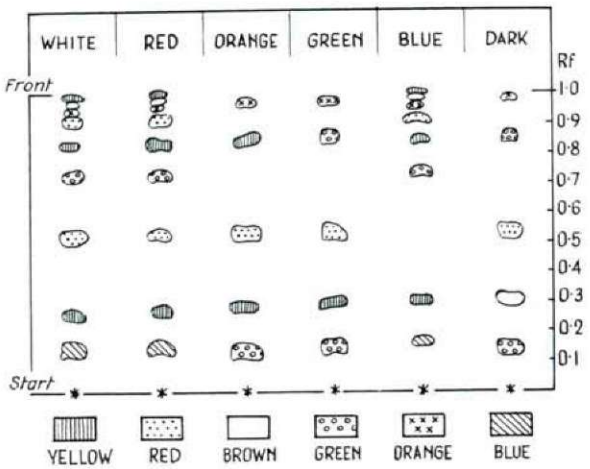

Fig. 9. 14-day *Aspergillus versicolor* pigments separated on a silica gel thin layer.

There appears to be a generally valid tendency for the pigment content to be poorer in orange and green lights and in the dark, whereas it is the most varied in white or red lights. The pigment compositions of the *Aspergillus* species are more varied than those of the *Penicillium* species.

It is characteristic for the individual species that they give a definite colour to the culture medium in their environment, this pigment being synthetized and excreted by the mycelium before or simultaneously with the spo-

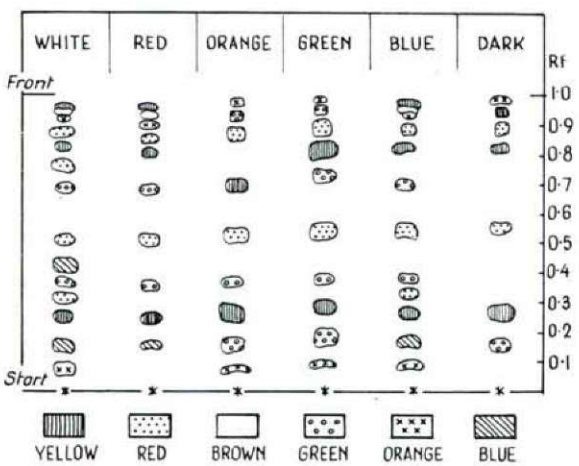

Fig. 10. 24-day *Aspergillus versicolor* pigments separated on a silica gel thin layer.

rulation. The pigment-synthesis and excretion by the mycelia is continuous, for the pigmentation of the nutrient solution becomes more intense.

The complexity of the absorption spectra indicate that the apparently homogeneous pigments produced by the mycelia are mixtures of several components. For technical reasons we have not yet been able to make a separate study of the pigments of the conidia, and so it cannot be estableshed from the chromatograms which parts of the fungus the individual pigments are derived from. The aim of our further studies is to separate the pigments if possible according to organs and to identify the individual organs chemically.

Effect of the composition of the culture medium on the pigment-synthesis

Change of the composition of the culture medium also leads to changes in the composition of the pigments.

Absorption spectra were taken in accordance with the fractions described in the methological part. The extenctions of fraction I of the pigments excreted into culture media of various compositions are shown in Figure 11. It can be seen that the excreted pigments are composed of several colour-components, the absorption maxima of which lie in the wavelength region 400–500 nm. The nature of the curve is the same in the case of the different culture media.

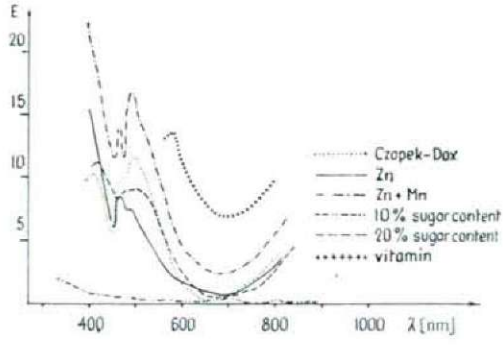

Fig. 11. Extinctions of *Penicillium purpurogenum* pigment extract after growth in nutrient solution of various compositions.

It appears that if a microelement such as Zn is added to the culture medium the value of the extinction decreases, i.e. the amount of pigment diffusing out into the nutrient solution decreases. The joint application of Zn and Mn promotes the excretion. As we have seen, the synergistic effect of the two microelements is also manifested in the growth of the mycelium.

With the increase of the sugar content of the culture medium the character of the curve does not alter, but in contrast the diffusing out of the pigment is inhibited. In sugar-rich culture media the bulk of the pigments is found in fractions II and III.

It is assumed that there is a relation between the increase of the sugar concentration and the retention of the pigment, presumably as a result of the change of the permeability or the stronger bonding of the pigments. However, the relation is not known.

References

CARLILE, M. J. (1965): The photobiology of Fungi. — Annual Review of Plant Physiology *16*, 175—199.

CARLILE, M. J. and J. FRIEND (1956): Carotenoids and reproduction in *Pyronema confluens*. — Nature *178*, 369—370.

EBERHARD, D., W. RAU und C. ZEHENDER (1961): Über den Einfluss des Lichts auf die Carotinoidbildung von *Fusarium aqueductum*. — Planta *56*, 302—308.

FFIEDERICKSON, I. and H. ENGEL (1960:) Der Abschussrhytmus der Fruchtkörper von *Sphaerobulus stellatus* (THODE) PERS. — Planta *55*, 313—326.

GARTON, G. A., T. W. GOODWIN and W. LIJINSKY (1951): Studies in carotenogenesis. 1. General conditions governing β-carotene synthesis by the Fungus *Phycomyces blakesleeanus*

GARTON, G. A., T. W. GOODWIN and W. LIJINSKY (1951): Studies in carotenogenesis. NTAJA BURGEFF. — Biochem. J. *48*, 154—163.

JOHNSON, T. W. and J. E. HALPIN (1952): Influence of light on the morphology and production of conidia in some species of *Dermatiaceae*. — Phytopath. *42*, 342.

MOHR, H. (1961): Wirkungen kurzwelligen Lichtes. In Handbuch der Pflanzenphysiologie, XVI. 439—531 (RUHLAND, W. Ed., SPRINGER, Berlin).

MUNTANJOLA, M., M. CVETKOVIČ and M. NESKOVIČ (1968): Studies on the effect of some substances as possible substitutes of light in the sporulation of *Aspergillus flavus* 28—A. — Bull. Inst. et Jard. Bot. Univ. Belgrade 2 n. s. 35—41.

MUNTANJOLA, M., M. CVETKOVIČ, M. NESKOVIČ and L. J. CULAFIC (1968): Morphogenetic responses of *Aspergillus aureolatus*. — Bull. Ints. Jard. Bot. Univ. Belgrade, 3 n. s. 29—34.

NARASIMHARACI, N. and L. C. NINING (1963): Studies on the pigments of *Penicillium herquei*. — Canadian J. Chem. *41*, 641—648.

RAPER, K. B. and C. THOM (1949): A manual of the *Penicillia* Baltimore.

RAPER, K. B. and C. THOM (1949): A manual of the *Aspergilla* Baltimore.

STAHL, E. (1967): Dünnschicht-Chromatographie. — Springer Verlag, Berlin.

SZALAI, I. és FRENYÓ, V. (1962): Növénytani praktikum II. Tankönyvkiadó, Budapest.

VÖRÖS, J. és G. UBRISZY (1960): A penészgombák. I. 8. Budapest.

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ON THE MECHANISM OF AUXIN – GIBBERELLIN INTERACTION VI. GIBBERELLIN-INDUCED CHANGE IN THE ACTIVITY OF IAA-FORMING ENZYME PREPARATAIONS

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Introduction

We have demonstrated in previous papers (VARGA, 1968; VARGA *et al.*, 1968) that after feeding the bean hypocotyl tissues with triptophan (TTP) and ^{14}C–2–TTP, the conversion of TTP to indoleacetic acid (IAA) or ^{14}C–IAA *(in vivo)* takes place in a higher degree in the GA-treated stems than in the untreated controls. It seems, therefore, that the utilization of the precursor in the IAA-biosynthesis is increased by GA.

It is anyway to take into consideration that at investigating the TTP ↑ IAA conversion *in vivo,* we could not follow simultaneously with attention and filter out several metabolic processes that can exert influence on the auxin yields. In case of *in vivo* experiments, for example, there was no possibility of gathering information about the degree of the oxidative degradation going on parallel with the IAA-synthesis. The concentration of IAA, however, measured at the given date, depends in a high degree upon the auxinoxidase activity. We regarded, therefore, desirable to carry out also the *in vitro* investigations of similar aim with cell-free enzyme preparations, for confirming the results of the *in vivo* experiments.

It has been proved in more works that cell-free enzyme preparations can perform the TTP → IAA conversion. GORDON wrote first in 1958 about an enzyme system extracted from shoot tips of *Phaseolus aureus* seedlings which catalyzed *in vitro* the process of the conversion of TTP to auxin. LANTICAN and MUIR (1967) described an IAA-producing enzyme system isolated from the apex of *Avena* coleoptiles, MOORE and SHANER (1967) from that of pea, VALDOVINOS *et al.*, (1967) similarly from that of pea and *Coleus,* and PHELPS and SEQUEIRA (1967) from that of tobacco shoots.

The degradation of TTP to auxin by cell-free plant tissue extracts can, therefore, be carried out *in vitro* too; and in the present experiments the effect of GA on the TTP → IAA conversion was investigated in this way, too. On the basis of the works of GORDON and PALEG (1961), GORDON and BUESS (1963) – who had referred to the TTP-degradation taking place spontaneously, as well, in the reaction mixtures – in the course of the experiments we took inti consideration the possible formation of IAA both in enzymatic and in non-enzymatic way.

Materials and Methods

The apical segments of seven days old shoots of bean seedlings (*Phaseolus vulgaris* L. var. Golden Rain), with the terminal bud and primordial leaves, were used for the experiments.

The cell-free enzyme preparation was made according to MOOR and SHANER (1967): the chilled tissues were homogenized with Mac Ilvain's buffer of double amount (pH 6.4 and 7.6), and after filtration were centrifuged with 105 000 x g for 90 minutes. In some cases the enzyme extract was dialyzed against 0.1 M buffer, for twenty hours, at 2 to 4°C.

With the enzyme preparation — in cases of both pH — four kinds of reaction mixtures were made, for the following aims:

1) Investigation of the enzymatic degradation of TTP in the presence of GA: 5 ml enzyme preparation + 5 ml TTP ($5 \cdot 10^{-4}$ M) + 10 ml GA (50 ppm).

2) Control of No. 1. without GA: 10 ml enzyme preparation +5 ml TTP ($5 \cdot 10^{-4}$ M) +10 ml buffer.

3) Investigation of the spontaneous conversion of TTP in the presence of GA: 5 ml preparation + 5 ml TTP ($5 \cdot 10^{-4}$ M) + 10 ml GA (50 ppm).

4) Control of No. 3 without GA: 10 ml TTP ($5 \cdot 10^{-4}$ M) +10 ml buffer.

The incubation took place two hours long in the dark, with a continuous current of air. After stopping the reaction and acidifying to pH 2.8, the IAA content of the mixtures was shaken into peroxide-free ethyl-ether and separated with paper chromatography (Sch & Sch 2043 b paper, isopropanol — 7% ammonia-water 8 : 1 : 1 solvent). The quantity of IAA was compared partly on the basis of spot size and colour intensity obtained by Ehrlich's reagent and defined in relation to the standard series, partly by photometric measurement of the eluate of the chromatogram-spots at 280 nm. For expressing enzyme activity, the following formula was used: mμg IAA/min. x ml enzyme (i.e. IAA mμg produced in the reaction mixture in one minute and multiplied with the present ml enzyme). It is to be noticed that the incubation time of two hours means about the saturation level, the curve of the IAA yield being linear for 120—130 minutes. Referring the efficiency of the enzyme preparation to protein-N, also the specific activity was expressed: mμg IAA/min. x mg nitrogen.

Every investigation was carried out with two parallels, in four replications.

Results and discussion

The IAA-synthesizing ability of the cell-free enzyme preparation was studied with the four sorts of reaction-mixtures described in the Methods, at pH 6.4 and 7.6. The parallel work at both pH was considered necessary because

Table 1. GA-induced change in the IAA-synthesizing ability of cell-free enzyme extracts from seven days old bean shoots (pH 7.6).

Incubation mixtures		mμg IAA/min.	Enzyme* activity	Protein—N mg/ml	Specific** activity	Activity percentage
1	enzyme TTP GA	764.0	3820	1.12	4278	215
2	enzyme TTP	354.1	1770	1.12	1982	100
3	buffer TTP GA	86.5	432	1.12	484	24
4	buffer TTP	92.8	464	1.12	520	26

* mμg IAA/min. x ml enzyme

** mμg IAA/min. x mg protein—N

the *in vitro* functioning of the TTP → IAA converting enzyme system has – on the basis of our previous investigations and KÖVES's paper (1965) – its optimum between pH 7 and 8 and then there is practically no IAA-oxidase activity. On the other hand, pH 6.4, which is less favourable to IAA-formation, is the pH optimum of the IAA-oxidase in bean.

In the course of the experiments TTP → IAA conversion was observed, in a lower degree, in the enzyme-free reaction mixtures Nos. 3 and 4 as well; the demonstrable IAA amount was, however, in both cases ± identical. The spontaneous degradation of TTP to auxin was, therefore, by the presence of GA perceptibly not influenced (Fig. 1). All the more was noticed the stimulating effect of GA on the enzymatic IAA-formation; its presence namely strongly increased in the first reaction mixture the TTP-converting activity of the enzyme extract, that is to say, the yield of IAA. The results referring hereto are given in Table 1. The conclusions that can be drawn from the data can be accorded essestially well with MUIR's (1964) results of similar aim obtained, however, with another method and on other objects. It was namely observed by the author, too, that after GA-treatment the enzyme preparations from apical tissue of dwarf pea and from tomato ovary performed the conversion of TTP to auxin in a higher degree.

Fig. 1. Relative quantity of IAA formed in the reaction mixtures, on the basis of spot size and colour intensity (pH 7.6).
1, 2, 3, 4 = reaction mixtures

In the experiments, the GA given to the cell-free enzyme preparation at pH 7.6 increased the yield of IAA to be more than double (Table 2). Carrying out the investigations under completely sterile conditions and in the presence of 200 μg/ml penicillin and streptomycin, the yield of IAA did not decrease (Table 2); it is not probable, therefore, that the TTP → IAA conversion observed would be of bacterial origin, as published in the papers of some authors (LIBBERT and WICHNER, 1963; LIBBERT *et al.*, 1966; WINTER, 1966; WICHNER and LIBBERT 1968, etc.). Some participation of epiphytic bacteria in

Table 2. Effect of GA, dialysis, and antibiotica on the IAA-synthesizing ability of the cell-free enzyme preparations made from bean shoot.

Enzyme preparations	Presence of GA	Yields of IAA	
		mμg/g fresh weight	μmoles/g fresh weight
Non-dialyzed	—	71.1	406
	+	152.7	872
Dialyzed	—	102.3	584
	+	211.6	1208
Non-dialyzed + penicillin and streptomycin	—	74.0	422
	+	148.9	850
Dialyzed + penicillin and streptomycin	—	100.7	575
	+	215.8	1233

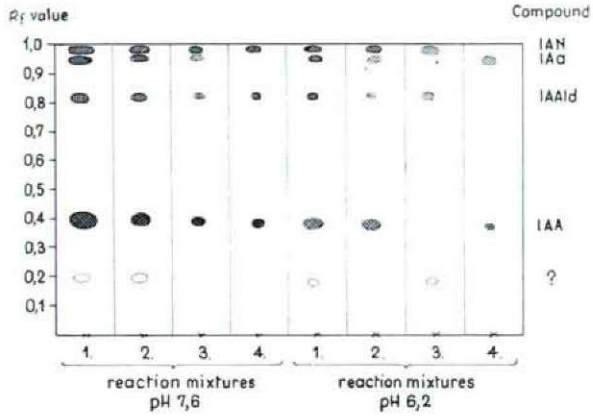

Fig. 2. Chromatogram-spots of IAA and IAA-precursors formed from tryptophan in the reaction mixtures.
(Solvent: isopropanol — 7% ammonia — water 8 : 1 : 1)

the IAA-production is, of course, not excluded completely by our experiments. But we do not believe that this could be considerable in this case – or in other similar experiments – as compared with the IAA-production of the enzyme system of apical stem tissues.

On the other hand, the yield of IAA was observably increased in the reaction mixtures, containing GA or not, by the dialysis of the enzyme preparation (against 0.1 M buffer, for 20 hours, at 2 to 4 °C). That can be explained by the removal of the compounds disturbing the reaction, resp. of the phenolic co-factors stimulating the IAA-oxidase activity.

For determining also the simultaneous IAA-destruction in the reaction mixtures, IAA of known quantity was added to the enzyme preparations (pH 7.6) and after two hours incubation the remaining auxin concentration was measured (VARGA and BÁLINT, 1966). The IAA-destruction proved to be of very low degree: 1.35 to 1.80 μg/h per ml of enzyme. That means that the

IAA could be regained in 92 to 94 per cent. In the tissue extract of the bean shoot applied, at pH 7.6, is therefore no considerable IAA-oxidase activity. The values given in Table 1 can therefore be considered as the actual auxin yields during the time unit.

The IAA quantity produced at pH 6.2 – although its presence was doubtless observed in the Nos. 1 and 2 reaction mixtures – was much less than that obtained at pH 7.6. That is obviously because in this case the *in vitro* TTP → IAA conversion of lower degree was followed by IAA-destruction of higher degree. The auxin-oxidase activity of the enzyme preparation was here 10.8 to 11.7 μg/h per ml of enzyme. This corresponds to regaining not more than 45 ± 3 per cent of the IAA added.

On the chromatograms of the ether extracts of the reaction mixtures, apart from the IAA spot, there could be observed, of course, other spots of indole compounds formed from TTP as well; and at pH 6.2, too, about in the same amount as at pH 7.6. From among these IAA precursors at R_f 0.83 indoleacetaldehyde (IAAld), at 0.92 indoleacetamide (IAa), and at 0.98 indoleacetonitrile (IAN) could be identified. Identification took place on the basis of R values, colour reactions, UV fluorescence and UV absorption spectrum, as compared to those of authentic compounds.

The result of the *in vitro* experiments carried out with cell-free tissue extracts have therefore confirmed again our previous statement that in bean shoots the biosynthesis of IAA from TTP is mainly realized through IAN – IAa, but another pathway through indolepyruvic acid (IPyA) – IAAld can also be proved (VARGA *et al.*, 1968; VARGA, 1971). It seems that the presence of GA exerts a stimulatory effect on these biochemical processes.

Summary

The ability of the cell-free enzyme preparations from apical tissues of bean shoots to realize TTP → IAA conversion was studied, in presence and absence of GA. Addition of GA to the enzyme preparation did not exert any influence on the spontaneous degradation of TTP to auxin; it did however considerably stimulate the enzymatic IAA production and multiplied many times the yields of auxin. The IAA-synthesizing ability of the tissue extracts was therefore definitely increased by the presence of GA. These *in vitro* experiments have confirmed the results of our earlier *in vivo* investigations.

Carrying out the experiments under sterile conditions and in presence of antibacterial compounds, the yield of IAA did not decrease. The TTP → IAA conversion observed cannot be, therefore, of bacterial origin.

References

GORDON, S. A. (1958): Intracellular localization of the tryptophan-indoleacetate enzyme system. — Plant Physiol. *33*, 23—27.

GORDON, S. A.—BUESS, E. (1963): Inactivity of hexitols and hexose in biosynthesis of auxin from tryptophan. — Plant Physiol. *38*, 11.

GORDON, S. A.—PALEG, L. G. (1961): Formation of auxin from tryptophan through action of polyphenols. — Plant Physiol. *36*, 838—845.

KÖVES, E. (1965): Adatok a természetesen előforduló fenolkarbonsavak növekedésszabályozó hatásának mechanizmusához. — Kandidátusi értekezés. (Thesis).

LANTICAN, B. P.—MUIR, P. M. (1967): Isolation and properties of the enzyme system forming indoleacetic acid. — Plant Physiol. *42*, 1158—1160.

LIBBERT, E.—WICHNER, S. (1963): Trytophan-Abbau zu Indolessigsäure durch pflanzenbesiedelnde Mikroorganismen. — Naturwiss. *50*, 451.

LIBBERT, E.—WICHNER, S.—SCHIEWER, U.—RISCH, H.—KAISER, W. (1966): The influence of epiphytic bacteriae on auxin metabolism. — Planta *68*, 327—334.

MOORE, T. C.—SHANER, C. A. (1967): Biosynthesis of indoleacetic acid from tryptophan—C^{14} in cell-freee extracts of pea shoot tips. — Plant Physiol. *42*, 1787—1796.

MUIR, R. M. (1964): Conversion of trytophan to auxin by plant tissue preparations. — Plant Physiol. *39*, Suppl. XVII.

PHELPS, R. H.—SEQUEIRA, L. (1967): Synthesis of indoleacetic acid via tryptamine by a cell-free system from tobacco terminal buds. — Plant Physiol. *42*, 1161—1163.

VALDOVINOS, J. G.—ERNEST, L. C.—HENRY, E. W. (1967): Effect of ethylene and gibberellic acid on auxin synthesis in plant tissues. — Plant Physiol. *42*, 1803—1806.

VARGA, M. (1971): On the mechanism of gibberellin — auxin interaction. VII. Effect of gibberellic acid on the utilization of auxin precursors in indoleacetic acid synthesis. — Acta Biol. Szeged *17*, 113—118.

VARGA, M.—BÁLINT, I. (1966): The effect of gibberellin on the growth, indoleacetic acid content and on the activity of indoleacetic acid oxidase in rice seedlings. — Acta Biol. Hung. *16*, 243—253.

VARGA, M.—KÖVES, E.—SIROKMÁN, F.—BITÓ, M. (1968): On the mechanism of gibberellin — auxin interaction. III. Effect of gibberellin on the biosynthesis of IAA from tryptophan. — Acta Bot. Hung. *14*, 435—442.

WICHNER, S.—LIBBERT, E. (1968): Interactions between plants and epiphytic bacteria regarding their auxin metabolism. I. Detection of IAA-producing epiphytic bacteria and their role in long duration experiments on tryptophan metabolism in plant homogenates. — Physiol. Plant. *21*, 227—241.

WINTER, A. (1966): A hypothetical route for the biogenesis of IAA. — Planta *71*, 229—239.

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ULTRASTRUCTURE INVESTIGATIONS ON THE CEREBRAL CORTEX OF THE SAND-LIZARD (LACERTA AGILIS L.)

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Introduction

The proper cerebral cortex appeared first at lizards although there are in the dorsal part of the forebrain of Amphibia some cell groups that may be considered as the precursors of the cerebral cortex.

In the cerebral cortex of lizards, going from the lateral wall of ventricle towards the surface of brain, the first layer is that of ependyma cells consisting of a single cell line. The cells are cylindric. Every cell is continued in an ependymal fibre that, going on upwards in the cerebral substance, is ramifying abundantly. The rami pass through the single layers and end, in the form of terminal heads, on the surface of brain. The ependymal fibres appear in the preparations impregnated according to GOLGI's method in so large numbers that cover the other layers of cortex almost entirely. The layer of ependyma cells is followed by the medullary layer.

The medullary layer is consisting of myelinated fibres. These are partly the neurites of the nerve cells of cortex, partly centripetal fibres originating from other cerebral regions, mainly from the olfactory region and ending in the cortex. The medullary layer is followed by the layer of pyramidal cells.

The pyramidal cells are forming more layers. They have got their name from their pyramid-like shape. Their cell body is triangular. The base of triangle is looking towards the cerebral ventricle. The cell part lengthened and directed towards the surface of brain is proceeding to a thick dendrite, the so called peak-dendrite ramifying abundantly. The rami becoming more and more thin enter the external molecular layer and end there. There originate from the base of pyramidal cells at about the middle region the neurite and from the corners the two basal dendrites. The latter ones are abundantly ramifying and their strongly thinned terminal rami develop the deep molecular layers (KRAUSE, 1921).

In the layer of pyramidal cells, apart from the typical pyramidal cells, there are other cell forms, too, of course, in a much lower number than the pyramidal cells. Cell forms like this are the triangular cells the peak of which is directed towards the ventricle of the brain and their two basal main dendrites towards the surface of the brain. Among the pyramidal cells there are bipolar cells, as well, occurring mainly in the dorsal and lateral regions of cortex. The layer of pyramidal cells is followed by the external molecular layer.

The external molecular layer consists overwhelmingly of terminal rami and centripetal fibres originated from the ramification of peak dendrites. In the layer, in addition to the fibres resp. fibre terminations, there can rarely be observed bipolar cells of rather large extent, as well, the longitudinal axis of which is parallel with the surface of brain. The outmost part of cortex is the tangential layer.

The tangential layer consists of fine unmedullated fibres running parallel with the cerebral surface. A number of the fibres are centripetal fibres, others of them are the rami of the neurites originating from the pyramidal cells. The neurites of not all the pyramidal cells are namely directed towards the medullary layer, but there are also that are bending back, stepping in the external molecular layer, in that ramifying and the rami ending in the tangential layer.

For recognizing the ultrastructure of the cerebral cortex of lizards, we have carried out electron microscopic investigations on the cerebral cortex of the sand-lizard.

Materials and Methods

For being investigated, small pieces were excised from the dorsal part of telencephalon, fixed in osmic acid buffered according to Milloning, dehydrated in alcohol of gradually increasing concentration and embedded in araldit. We made sections from the material with an L.K.B.-ultramicrotome and investigated them with electron microscopes TESLA D 242 and JEM 6. Some of the investigations were performed in the Biological Research Institute of the Hungarian Academy of Sciences at Tihany others in the electron microscopic laboratory of the Institute of Biochemistry, University Medical School, Szeged. In the following we sum up the results of our observations concerning some components of the cortex.

Nerve cells

There are characteristic for the nerve cells: the narrow, margin-like cytoplasm (pericarion), a round nucleus of central position, as well as the processes, neurite and dendrites. In the cytoplasm, the endoplasmic reticulum, that is to be regarded as the largest organellum of the cell, appears in the form of various cisternal systems. Its shape and external form, the extent of the single cisterns, its course, connection in the various cells are very different, showing in some case a peculiarly different picture. There are cases where some parts of cisterns widen out extremely, forming very large cavities. On other occasions, the cavities resp. cavity systems of straight course, uniform in length and quasi identical in diameter, too, arranged beside each other are showing a form reminding us of the chords of lute. There are cases when the tubes of reticula are of proportionally narrow lumen, showing anyway in some places smaller or larger dilatations. The cisternal regions developed in this way, in which the dilatations and strictures are alternating with each other, take on a wavy shape and the tube systems that are parellel with one another are forming specific wave systems. Between the cisterns there appear sometimes some roundish formations limited by thin walls in the central substance of which there may be observed very small granules being electron-dense only in a low degree. (Fig. 1).

In the cytoplasm a particular place is taken by GOLGI's complexes. We have to say that in the course of our investigations covering almost every region

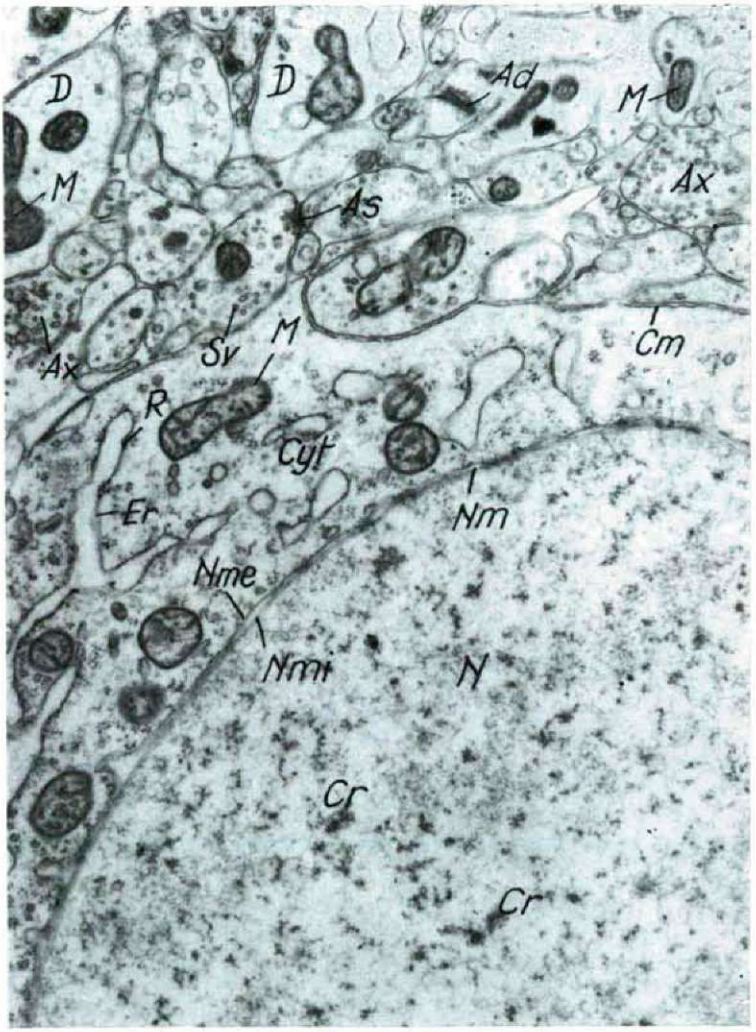

Fig. 1. *Lacerta agilis*. Cerebral cortex. Nerve cell. Cyt — cytoplasm, Cm — cell membrane, N — nucleus, Nm — nuclear membrane, Nmi — internal nuclear membrane, Nme — external nuclear membrane, Er — endoplasmic reticulum, M — mitochondrium, R — ribosoma, Cr — chromatin, Ax — axon, D — dendrite, Sv — synaptic vesicle, As — axo-axonic synapse, Ad — axo-dendritic synapse. Magnified: x 25.00.

of the brain we have found nowhere as many GOLGI's complexes as in the cerebrocortical nerve cells of the sand-lizard. There are microscopic pictures in which 5 to 6 and even more GOLGI's complexes can be observed showing interesting and specific formations. It is generally characteristic of these that the vesicular groupings are richer and more developed than the tubular region. The vesicles are comparatively large, showing a perfectly round form, and the many empty vesicles of equal size are followed at the edge of the field by

large electron-light cysts. The high number and conformity of vesicles, the association of vesicles of large size, the abundance in short tubes and cavity systems speak in the favour of the supposition that at lizards GOLGI's cerebral complexes may have a peculiar role. It is, of course, possible, too, that these marks do appear just at the sand-lizard that is extremely sensitive and moves incredibly fast (Fig. 2).

In the cytoplasm, both in the pericarion and in the dendrites, the multivesicular bodies appear not in a high number but in a sharp form and in a

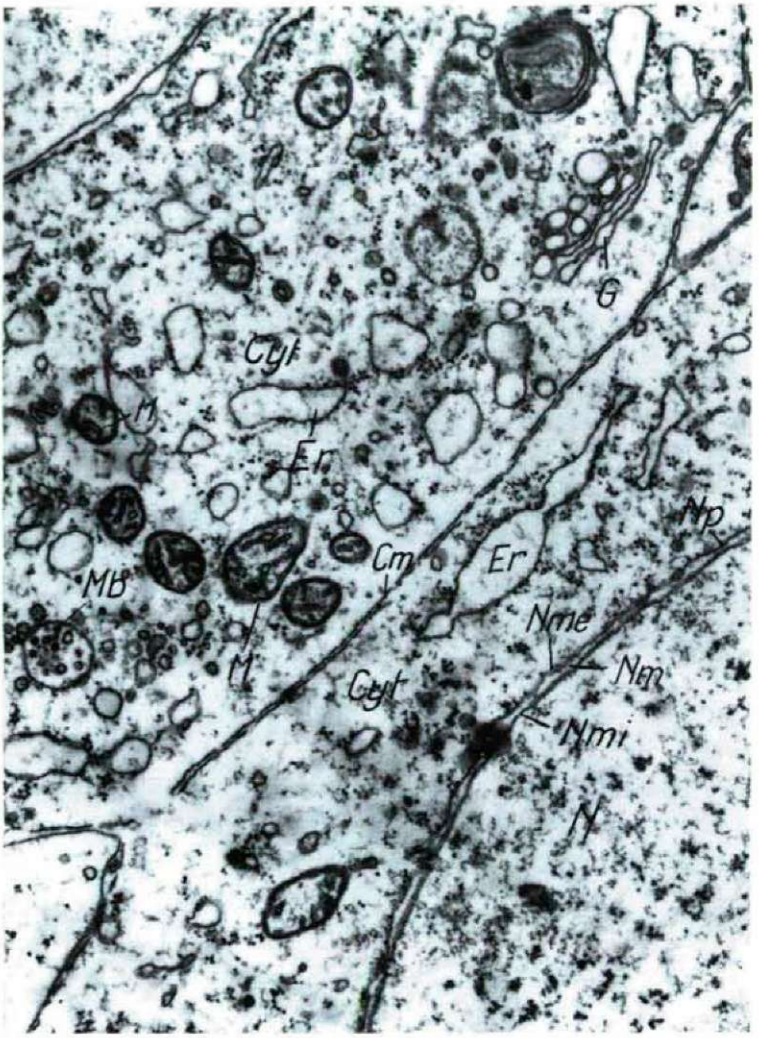

Fig. 2. *Lacerta agilis*. Cerebral cortex. Nerve cells. Cyt — cytoplasm, Cm — cell membrane, G — Golgi's complex, Mb — multivesicular body, Er — endoplasmic reticulum, M — mitochondrium, N — nucleus, Nm — nuclear membrane, Nmi — internal nuclear membrane, Nme — external nuclear membrane, Np — nuclear pore. Magnified: x 30.000.

comparatively large size. They are generally roundish or long-shaped, their wall is thick, homogeneous, and within them, limited from one another well, several vesicles of comparatively equal size may be seen. The latter ones do not touch one another, their stroma is of loose construction.

In the cytoplasm, ribosomes can be seen in large numbers. Their locations and groupings are different. There are some microscopic pictures in which the ribosomes arranged in lines beside the endoplasmic cisterns are following exactly the course of the latter ones. Besides these pictures there are some others, too, and not rarely, in which the ribosomes form groups, and even the general situation is that in the same picture both formations are to be found. In the middle of the groups the ribosomes are nearly touching one another but near the border of the group they become strongly rarified.

Some characteristic components both of pericarions and of processes are the mitochondria. They belong to the crista type. In their structure we can distinguish well the double mebrane and the central matrix in which the cortex of the crests engendered by the invagination of the internal membrane and the central light part always appear sharp. Between the crests we see here as well as generally in the mitochondria everywhere an electron-light matrix substance. The shape of mitochondria is extremely interesting and various. Most of them are manifesting the usual ellipsoid resp. elliptical forms although there occur sporadically also those of them that may be included in the spheric type. Apart from them, the peculiar forms are not rare, either. There are remarkable those showing abnormity in width or length. Mainly the longitudinal overgrowth seems to be a rather frequent appearance. The shapes of a crescent or of an expressed shepherd's crook are not rare, either. There are, however, rare the forms referring to a division. The shape, size and direction of crests is manifold, their grouping is peculiar (Fig. 3).

The cell nucleus is generally big, it constitutes almost three-quarters of the cell. It is an electron-dense granulated body, surrounded only by, a very narrow pericarion border. Its substance is loose, the chromatin clots are formless, forming sporadically larger knots. In the nucleus is to be found the nucleolus, usually of excentric location. Its shape is irregular, sometimes roundish. Its substance is much more electron-dense than that of the nuclens, consisting of round knots arranged close to one another. The nucleus is surrounded by a double nuclear membrane distinctly visible. The area between them is proportionately wide and in every case sharply conspicuous. The two nuclear membranes – here as well as generally – adhere sporadically close to each other, later however getting farther. In these places the nuclear pores are to be seen. It is not rare either, that the external nuclear membrane gets into a great distance from the internal one, folding into the pericarion. As the process repeats in the course of the external nuclear membrane, peculiar cavity systems develop called herniae of the nuclear membrane.

The neurite is a thin process of an unvarying thickness, limited sharp by the highly consipicuous but proportionately thin axolemma towards the adjacent tissue components. In the axoplasm we can see, even if not always, the neurofilaments of various thickness, located generally longitudinally. Among the latter ones there are some, as well, the course of which is not parallel with that of the axis of axon. We have come to this conclusion from the fact

that in microscopic pictures they appear in cross-section. The synaptic vesicles are special ingredients of neurites. These are empty vesicles of 150–300 Å diameter that, as proved by our pictures, fill in completely not only the axon terminals but also other axon regions having no synapses at all. Such a mass of vesicles never occurs in dendrites. This mark enables us to distinguish the dendrites of various sizes from neurites and to find our way in the different forms of the synaptic connections, resp. contacts.

Dendrites are cellular processes of the most various size and dimension. Their structure is agreeing with that of pericarion. Their substance is loose.

Fig. 3. *Lacerta agilis*. Cerebral cortex. External molecular layer. Ax — axon, D — dendrite, V — vesicle, M — mitochondrium, Ad — axo-dendritic synapse, Iv — invaginative synapse, Mb — multivesicular body. Magnified: x 25.000.

They are characterized by the neurotubules located longitudinally and transversely, and by microvesicular bodies. The synaptic vesicles are completely missing. The diameter is variable. The limiting membrane (dendrolemma) is very consipcuous well limited electron-dense membrane. In the dendroplasm there are to be seen sporadically major electron-light vesicles too (Fig. 4).

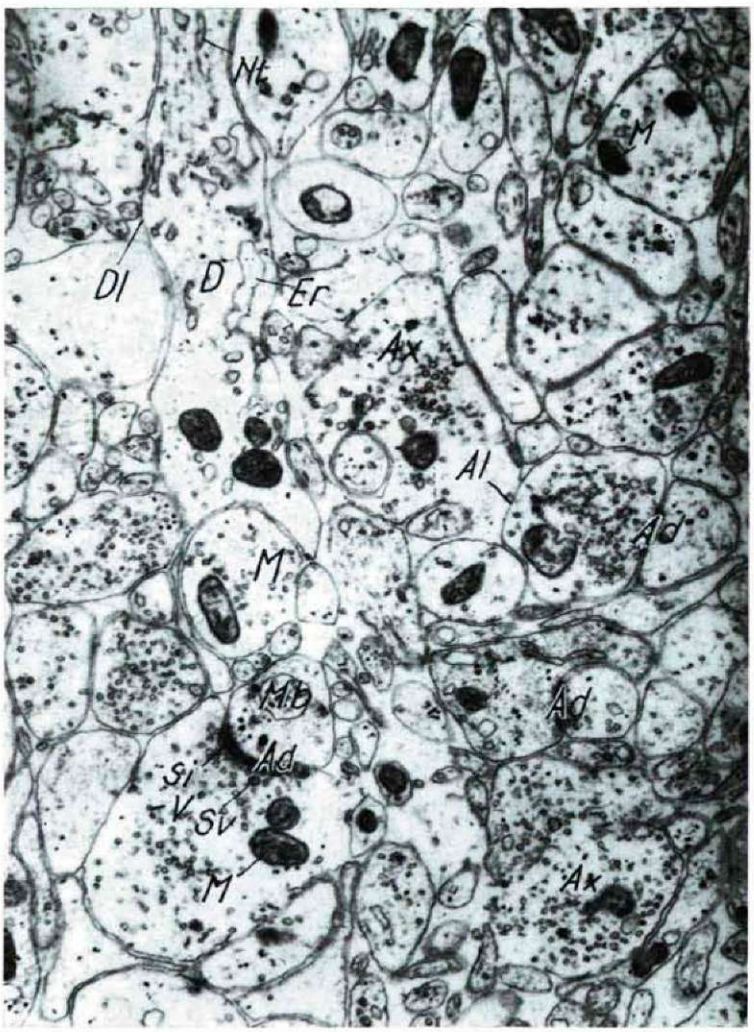

Fig. 4. *Lacerta agilis*. Cerebral cortex. External molecular layer. Ax — axon, D — dendrite, Al — axolemma, V — vesicle, M — mitochondrium, Er — endoplasmic reticulum, Mb — multivesicular body, Ad — axo-dendritic synapse, Si — intersynaptic space, Sv — synaptic vesicle, Dl — dendrolemma, Nt — neurotubule. Magnified: x 25.000.

Glia cells

They are structurally close to the nerve cells (ROBERTIS, GERSCHENFELD and GOMEZ, 1961). In the pericarion there are to be seen about the same cell organella as in the nerve cell. Characteristic differences are manifested in form and arrangement of the cisternae of the endoplasmic reticulum. The tubules are ramifying, their course is irregular. Multivesicular bodies appear often, the number and location of the vesicles differing highly. There is a very great difference in the arrangement of ribosomes. The latter ones are forming groups that are not limited sharp from one another. Every group has a centre where there are more ribosomes, and a peripheral part where the number is strongly diminished and the single shapes are far from one another. Sometimes the cytoplasm is so much full of ribosomesö that it seems to be a real granulated electron-dense substance divided into peculiar pieces by the endoplasmic cisternae and GOLGI's complexes forming peculiar systems. The accumulation of glycogenic clots is characteristic of glia cells. The situation is, namely, that glycogen is transported for the nerve cells, resp. neurons by glia cells.

The nucleus of glia cells is showing, as well, some characteristic feature. This manifests itself first of all in the fact that the electron density is fainter than in the nerve cells and round the internal nuclear membrane there are no circularly located polymorphous electro-dense areas. It is to be considered as characteristic, as well, that the nucleus of glia cells is long-shaped and the two nuclear membranes are very close to each other. The hernia-like protrusions that always occur in nerve cells can in glia cells never be found.

The glia fibres are as much characterless as the glia cells are. They are usually thick, the rami are rougher and the substance within the glia membrane is homogeneous. In that, there are to be seen neither any filaments nor any vesicles, although in some cases in the apparently empty substance some vesicles of larger dimension can be observed connected with one another with thin canal pieces.

Synapses

The synaptic contacts are characteristic of the cerebral cortex and, within it, first of all of the fibrous layers. Their number is generally high. Four to six and possibly more synapses can be distinguished in each picture. They are structurally chemical synapses the components of which are, according to PALADE and PALAY (1954), ESTABLE, REISSING and ROBERTIS (1954), PALAY (1956, 1958), FERNANDEZ-MORAN and BROWN (1958), ROBERTIS (1955, 1958, 1959), LORENZO (1959), ROBERTIS and IRALDI (1961), WHITTACKER and GRAY (1962), LOOS (1963), WESTRUM (1966), JONES (1969), the presynaptic plasm, the presynaptic membrane, the synaptic space, the postsynaptic membrane and the postsynaptic plasm (Fig. 5).

The components of the presynaptic plasm are the presynaptic organella. There belong to them the synaptic vesicles, the mitochondria, as well as the neurofilaments and neurotubuli. From among the synaptic vesicles the empty vesicles appear in the largest quantity. Their size varies between 250 and 600 Å. It is interesting that not only the axon terminals are full of empty synaptic vesicles but also the parts of axons that are not in synapsis.

Fig. 5. *Lacerta agilis.* Cerebral cortex. Nerve-fibre layer. Ax — axon, D — dendrite, Al — axolemma, Dl — dendrolemma, Ad — axo-dendritic synapse, V — vesicle, Sv — synaptic vesicle, Pr — presynaptic membrane, Po — postsynaptic membrane, Cl — cluster, M — mitochondrium. Magnified: x 48.000.

Fullness is singularly characteristic of the cerebrum of this animal. It is not excluded, and even it seems to be probable, that the cause of this particular richness is, as already mentioned, the strong agility and high sensitivity of this animal. If the transmitter material is transferred by the empty vesicles then that is natural because they need for the fast movements many stimuli, and for releasing the way of the impulse conduction depolarizing the postsynaptic membrane they need much transmitter material. As soon as we have fixed the cerebral cortex of the sand-lizard we had the thought that here the vesicle system must be richer and denser than in other animals moving slower and more sluggishly. It is to be mentioned as a peculiarity that that some empty vesicles exactly of the same appearance as that of those filling

both the terminal and the praeterminal regions of axons may be observed in dendrites, too, anyway not in large numbers, only in a few cases. And even it occurs that these show some groupings in the immediate vicinity of the postsynaptic membrane. In the presynaptic plasm it is sometimes possible to see, apart from the empty synaptic vesicles, electron-dense vesicles of a thickness of 1000 Å, as well. Without going into functional explanations, we have to refer to that the dense-core forms occur everywhere and in every axon in the animal kingdom, in smaller or larger quantity, both in the synaptic region and in the presynaptic axon sections. In connection with the phenomenon, we should like to emphasize only, in addition, that the structure of these vesicles is the same in the animal kingdom everywhere and in each organ.

It is mentioned repeatedly that one of the characteristic features of the presynaptic protoplasm is that in it the mitochondria accumulate, forming a

Fig. 6. *Lacerta agilis*. Cerebral cortex. External molecular layer. Ax — axon, — D — denrite. V — vesicle, Sv — synaptic vesicle, Ad — axo-dendritic synapse, As — spine synapse, Pr — presynaptic membrane, Po — postsynaptic membrane, Si — intersynaptic space, M — mitochondrium, Cr — cristae mitochondriales, Mn — mitochondrial membranes, Cl — cluster. Magnified: x 48.000.

group close to the presynaptic membrane. This statement cannot be referred at all to our material. We had investigated a great many synapses and found so that the number of mitochondria varied between 0 and 3. Reckoning by and large, we have found numbers 0 and 1, to be equal the most of them being number 1. In a sense like this, in our case it is not to be thought of the accumulation of mitochondria.

In our pictures from among the organella of the presynaptic protoplasm the less obvious ones were the fibrous components of the axons, the neurofilaments. In our opinion, the cause of this is to be sought for in the fact that the pieces of these fibres are intermingled among the synaptic vesicles past recognition, the latter appearing in every case in very large numbers and unusually sharp.

The presynaptic membrane is an everywhere sharply separated, structurally homogeneous membrane. Thickening is alweys conspicuous. It is generally not thick. It is interesting and in our case chracteristic, too, that along a few longer presynaptic membranes there are smaller or larger interruptions in the thickness dividing the membrane into two or three parts. It is interesting in this division that in the presynapse the cumulation of vesicles, the so-called cluster is showing a double resp. triple division, as well (Fig. 6).

The synaptic space is a conspicuous, electron-light substance of the same breadth that shows, magnified weakly, no division or structure at all. In pictures, however, magnified rather strongly, it is full of parallel ridges perpendicular to the synaptic membranes. According to our observations, it is of the same brath in every synapse. In our material, the synapses cannot be classified on the basis of the synaptic space.

The postsynaptic membrane always shows some fringing towards the postsynaptic plasm, resp. it is not limited towards the central region of the postsynaptic plasm. In a great part of cases it is thicker than the presynaptic membrane (Fig. 7). This phenomenon was noticed first by GRAY (1959), in the course of investigating the synaptic linkages of the cerebral cortex. Later on, seeing that in a part of the cerebral synapses the synaptic membrane is thicker than the presynaptic one, in other ones however the thickening of both membranes is the same, he divided the cerebrocortical synapses into two groups. He arranged into the first group those with a thicker postsynaptic membrane, into the second one those with the same thickening of both membranes. At a later date, the former one was named the synapsis of type GRAY I, the latter one that of type GRAY II. Both forms were found in our material, too, although – after having observed the pictures magnified more strongly – it could be said, as well, that all the forms of synapses belonged to the type GRAY I. The situation is namely in almost every case that the postsynaptic membrane is much thicker, darker and the fring-elike appendages penetrate deep into the postsynaptic plasm. In most part of the cases it is so that in both forms of synapses one axon is in contact with one dendrite but is occurs, too, and even not rarely, that a axon piece is connected with two dendrites (Fig. 5).

The postsynaptic plasm is showing no peculiarty at all. The empty vesicles and the neurotubules do appear sporadically here, too, but the former ones in an extremely low number. Mitochondria are found in it only occasionally.

Axodendritic synapses

In our material, 95 to 96 and perhaps even more per cent of the chemical synapses appearing extremely sharp and in large numbers belong to the axo-dendritic forms. Their shape and size are highly different. Both are a result of meeting forms. There are cases when the synaptic endings getting into contact are by and large of the same form. The situation is, however, generally so that the axon terminal is of larger extent than the surface of dendrite that is in contact with it. There occur, anyway, some cases, too, when the dendrite surface is larger than the detail of axon. The junctional axon ending is

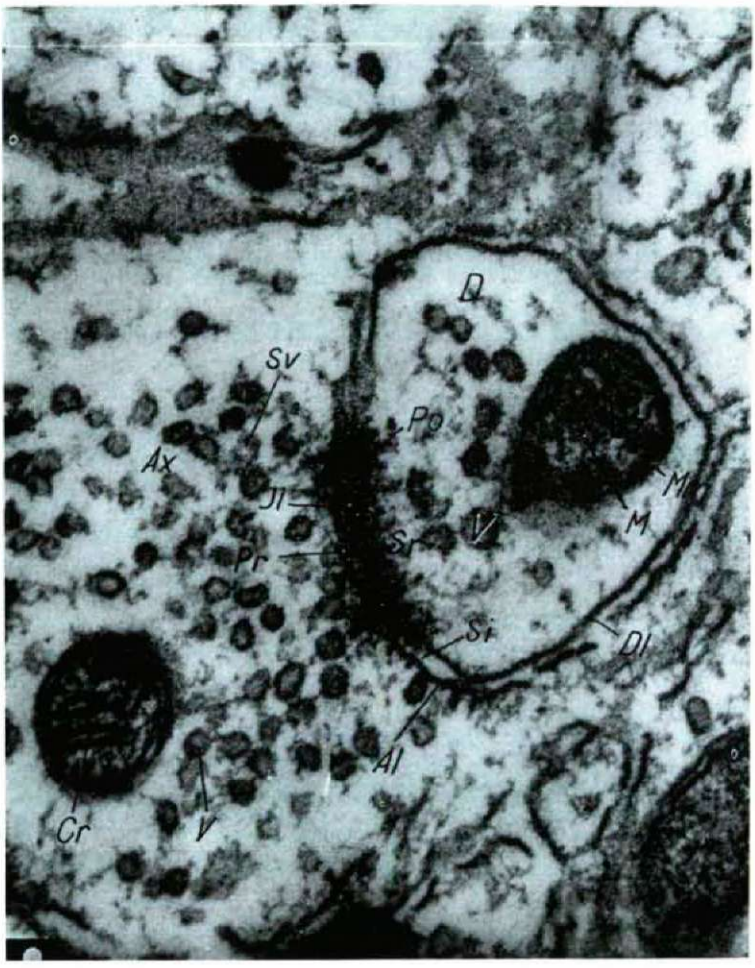

Fig. 7. *Lacerta agilis*. Cerebral cortex. External molecular layer. Axo-dendritic synapse. Ax — axon, D — dendrite, Al — axolemma, Dl — dendrolemma, V — vesicle, Sv — synaptic vesicle, Pr — presynaptic membrane, Si — intersynaptic space, Po — postsynaptic membrane, Sr — synaptic fringes, M — mitochondrium, Cr — cristae mitochondriales, Mm — mitochondrial membranes, Il — intersynaptic ridges. Magnified: x 100.000.

usually straight or rounded. In this case, it is embedded in the excavation of the dendrite. There are anyhow also some cases when the excavation is in the axon ending and in that is lying the dendrite ending or some part of the dendrite. There are interesting the synapses in which the side of a longer axon ending or that of a longer preterminal piece is in contact with a small dendrite ending or a preterminal piece of lesser extent of a dendrite. These pictures are favourable the opinion that the transmission of stimuli is not limited to the endings. That means that in the transmission of stimuli there can have a role from both parts some fibre-regions that are far from the endings.

A peculiar form of the axo-dendritic synapses appearing rather rarely is the invaginated synapse. It is a form of contacts when we see in the cross-

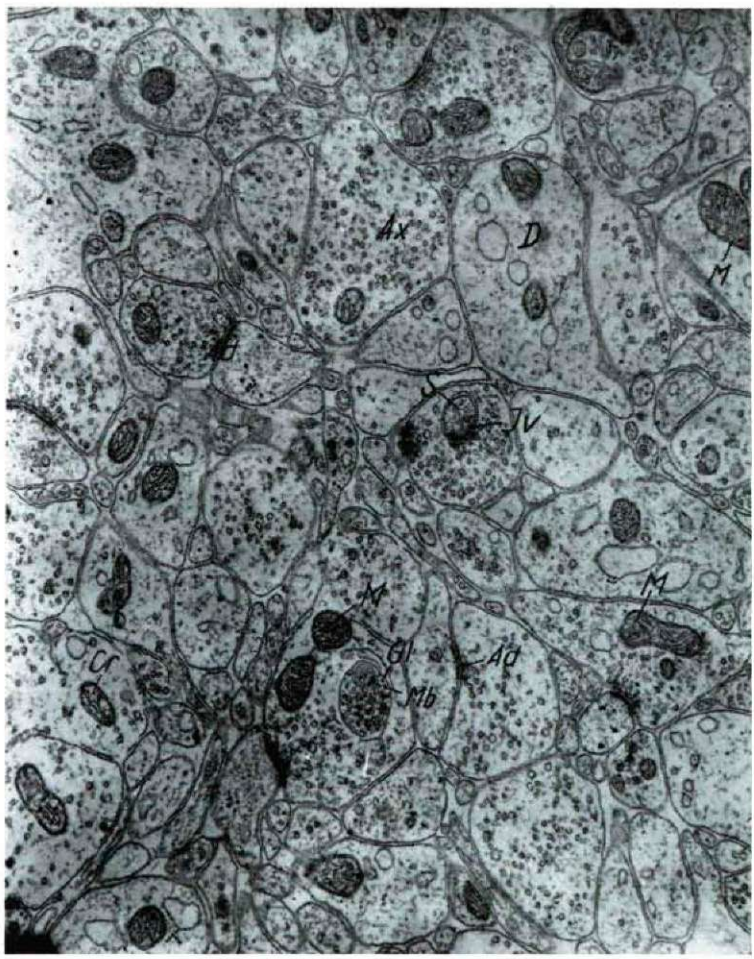

Fig. 8. *Lacerta agilis*. Cerebral cortex. External molecular layer. Invaginative synapse. Axo-dendritic synapses. Ax — axon, D — dendrite, S — dendrite-spine, M — mitochondrium, Mb — multivesicular body, Ad — axodendritic synapse, Iv —invaginative synapse, Cr — cristae mitochondriales, Gl — glycogen. Magnified: x 25.000.

section of a axon the cross-section of a dendrite piece with which the axon is forming synapse. But the same axon synaptizes with another dendrite piece, as well, that is lying outside the substance of neuraxon. According to our opinion, the matter in question is here an invaginated form of synapse that is forming a axon with a dendritic spine. The peculiar picture is the demonstration of a situation when the knife is cross-cutting a dendritic spine located in the cavity of an axon ending forming a synapse with

Fig. 9. *Lacerta agilis.* Cerebral cortex. External molecular layer. Synaptic vesicle, diapedesis. Ax — axon, D — dendrite, V — vesicle, Sv — synaptic vesicle, Cl — cluster, Pr — presynaptic membrane, Po — postsynaptic membrane, Si — intersynaptic vesicle diapedesis, Ad — axo-dendritic synapse, M — mitochondrium, Cr — cristae mitochondriales, Er — endoplasmic reticulum, Mb — multivesicular body. Magnified: x 25.000.

the spine. In our picture this would be the central synapse. The other one that in our case could be said to be a peripheric synapse does exist – as concluded from the situation – similarly between the axon and a dendrite spine. That may be concluded, apart from others from the fact, too, that round the peripheral synapse, and generally between the axon endings, there is everywhere a large number of smaller of bigger dendrite spines (Fig. 8).

Like of a peculiarity, we have to speak of an axodendritic form of synapses in which in the synaptic space, between two clusters a synaptic vesicle of large size is located. The situation is essentially that the presynaptic membrane is broken and in the space of the rupture, there is a larger vesicle in transition, pushing before itself the postsynaptic membrane. Supposedly, there is here a real diapedesis in the course of which the vesicle gets inside the dendrite. We cannot answer the question what is the cause of this peculiar phenomenon. It may be supposed that this vesicle of striking size is containing another material than the lesser ones ranging themselves along the membrane. It is, however, possible, as well, that it is containing the same material but more of it than the others do. At any rate, the phenomenon in interesting, the problem needs being clarified. This synaptic form is characterized, apart from the facts outlined above, also by that among the synaptic vesicles forms of dense-core type can be observed, as well (Fig. 9).

Axo-somatic synapses

Apart from the extremely numerous axo-dendritic synapses, we have found a few forms of synapses, too, where the axon is in synaptic contact with the soma of the nerve cell. These usually show the common form but we have found some of them, too, that – owing to its peculiar structure as well as to the peculiarity of its supposed function – will take some more explaining. In this synaptic form that belongs structurally to type GRAY II, there are two subsynaptic membranes parallel with and close to, each other, immediately under the postsynaptic membrane. The two membranes surround a narrow cavity along the whole length of the contact. One end of the canal is open, the other one is leading into an endoplasmic cistern (Fig. 10). The structure is unparalleled, unknown in the literature. To-day, when there is much discussion about the place, media and material basis of memory that can be seriously in question in this relation, we have the following ideas concerning the functioning of this system. Is it true, as said and written, that the basis of memory is protein, then our synapse may play the following part. The synaptic vesicles lining up along the presynaptic membrane in the axon terminal, with the acetylcholine contained by, resp. connected with, them – if that is, indeed, the transmitter in the cerebral cortex – make the postsynaptic membrane permeable and then the stimules is transferred to the subsynaptic canal, resp. to both subsynaptic membranes. From these it gets to the membrane of the endoplasmic cistern where it transmits informations to the ribosomes lined up there, then the protein production begins and together with that the corresponding change in the situation, quantity and the strength of memory. Nobody knows if it is so or not, at any rate, the idea seems undoubtedly to be plausible. That is confirmed by the structure, placing at disposal plenty of the supposed morphological bases for adventures of this kind.

Fig. 10. *Lacerta agilis.* Cerebral cortex. Axo-somatic synapse, Ax — axon, V — vesicle, M — mitochondrium, Ad — axo-dendritic synapse, As — axo-somatic synapse, Cyt — cytoplasm, Cm — cell membrane, Er — endoplasmic reticulum, Pr — presynaptic membrane, Sm — subsynaptic membrane, Ec — endoplasmatic cistern, Cc — subsynaptic canal, Sv — synaptic vesicle, Cl — cluster, R — ribosome. Magnified: x 48.000.

Axo-axonic synapses

In our material we have met this synaptic form extremely rarely. It occurred generally in the vicinity of nerve cells where the axon terminals becoming very thin form rich groups along the cell membrane. As to the structure of these synaptic forms, the situation is generally that from among the axon terminals running across one another, at the end of one of them there is an excavation and the end of the other one is rounded. The synaptic vesicles can be seen in both axon terminals scattered, sporadically in smaller groups distinctly visible. In the presynapse, the cluster manifests itself in a sharp form.

Capillaries

In connection with the ultrastructure of the cortex we have to discuss the structure of capillaries, as well, being essentially the same as anywhere in the organism of Vertebrata. Some characteristics of certain degree can only be observed in the shape of the endothelial cells and in their connection with one another. It may be called as a characteristic, apart from some others, that in the same capillary cross-section the endothelial cells appear in the most various shapes. On their surface, a great number of peculiar protrusions and hollows may be seen, that are showing, even in the same picture, a strongly varying form. The most characteristic parts of cells are the long processes hanging down into the lumen and showing, both in their shapes and situation, a great variety. In the cytoplasm, there are many empty round vesicles, arranged in a line. Complicated tubule systems are formed by the endoplasmic reticulum, and the mitochondria are forming groups. The ribosomes are arranged sporadically into lines, somewhere else they form undefinite and varying groups. The nucleus is a long-shaped, electron-ligth body. The chromatin clots form sporadically loose knots. The nuclear membranes are close to each other. The basal membrane is showing an oblique striation.

Summary

As a result of the ultrastructure investigations carried out in the cerebral cortex of the sand-lizard (*Lacerta agilis* L.) the following have been established.

1. The pericarion of the nerve cells is a narrow cytoplasm border, the cell membrane is sharp, the nucleus is big, roundish, the nucleolus is ovoid and of excentric location.

2. The cisterns of the endoplasmic reticulum are forming branchy systems. The vesicle grouping of GOLGI's complex is richer than the tubular region. There are many multivesicular bodies and ribosomes. Shape and size of the cristic mitochondria is strongly changing.

3. The substance of nucleus is loose, the chromatin clots are forming dispersed, irregular knots, the nucleolus consists of a large number of small, roundish granules. The nuclear membrane is double, the site and lumen of the nuclear pores is changing.

4. The glia cells are structurally close to the nerve cells. The tubules of the endoplasmic reticulum are ramifying, the multivesicular bodies are frequent, there are many ribosomes and glycogenous clots. The nucleus is longshaped, the space between the nuclear membranes is narrow.

5. The synapses are chemical synapses. The components manifest themselves completely and in a sharp form. They belong overwhelmingly to the axodendritic type but a low number of axo-somatic and axo-axonic forms may be observed, as well.

6. There are not rare among the axo-dendritic synapses the invaginative forms, either, in which the excavation of the axon terminal is forming a double synapse, one with the dendrite spine in it and another with a dendrite piece beside it.

7. Under the postsynaptic membrane of one of the axo-somatic synapses we have found a subsynaptic canal opening into the cistern of the endoplasmic reticulum. The structure, that is new for the literature, seems to be usable for the analysis of problems connected with the memory.

References

ESTABLE, C., REISSING, M., and ROBERTIS, E. De (1954): Microscopic and submicroscopic structure of the synapsis. — Exp. Cell. Res. *6*, 255—262.

FERNÁNDEZ—MORÁN, H. and BROWN, R. (1958): The submicroscopic organization and function of nerve cells. — Exp. Cell. Res. (Suppl.) Academic Press. New York.

GRAY, E. G. (1959): Axo-somatic and axo-dendritic synapses of cerebral cortex: an electron microscope study. — J. Anat. (Lond.) *93*, 420—433.

GRAY, E. G. (1959): Electron microscopy of synaptic contact on dendrite spines of the cerebral cortex. — Nature (Lond.) 1592—1593.

JONES, D. G. (1969): The morphology of the contact region of vertebrate synaptosomes. — Z. Zellforsch. *95*, 263—279.

KRAUSE, R. (1921): Mikroskopische Anatomie der Wirbeltiere in Einzeldarstellungen. — Berlin und Leipzig.

LORENZO, de A. J. (1959): The fine structure of synapses. — Biol. Bull. Woods Hole *117*, 390—399.

LOOS, H. van DER (1963): Fine structure of synapses in the cerebellar cortex. — Z. Zellforsch. *60*, 815—825.

PALADE, G. E. and PALAY, S. L. (1954): Electron microscope observations of interneuronal and neuromuscular synapses. — Anat. Rec. *118*, 335—336.

PALAY, S. L. (1956): Synapses in the central nervous system. — J. Biophys. Biochem. Cytol. (Suppl.) *2*, 193—202.

PALAY, S. L. (1958): The morphology of synapses in the central nervous system. — Exp. Cell. Research. Suppl. *2*, 275—293.

ROBERTIS, E. De (1955): Submicroscopic ogranization of some synaptic regions. An electron microscope study of the synapse. — Acta neurol. latino-americana *1*, 3—15.

ROBERTIS, E. De (1958): Submicroscopic morphology and function of the synapse. — Exp. Cell. Res. Suppl. *5*, 347—369.

ROBERTIS, E. De (1959): Submicroscopic morphology of the synapse. — Internat. Rev. Cytol. *8*, 61—66.

ROBERTIS, E. De and GERSCHENFELD, H. M. (1961): Submicroscopic morphology and function of glial cells. — Internat. Rev. Neurobiol. *3*, 1—65.

WESTRUM, L. E. (1966): Synaptic contacts on axons in the cerebral cortex. — Nature (Lond.) *210*, 1289—1290.

WHITTAKER, V. P. and GRAY, E. G. (1962): The synapse: biology and morphology. — Brit. med. Bull. *18*, 223—228.

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ANATOMICAL AND HITOLOGICAL OBSERVATIONS ON THE TRACHEAL GILL OF PALINGENIA LONGICAUDA OLIV. (EPHEMEROPTERA)

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The respiratory organ of the Ephemeroptera larvae, which is suitable for the respiratory metabolism of the aquatic animal, consists of tracheal trunks branching richly in the body, and of branchial lamellae arranged on both sides of the abdomen.

The anatomical observations previously reported on the respiratory apparatus of the Ephemeropterae extended to the study of both sections (LANG, 1913; SCHOENEMUND, 1929; LANDA, 1948). In addition to the macroscopic descriptions there are also important papers seeking phylogenetic correlations between the individual groups of the Ephemeropterae on the basis of the development and state of the respiratory organ and other systems (nervous system, Malpighian tubules) (LANDA, 1948; LANDA, 1969).

The paired branchial lamellae, which are situated on the 1st–6th abdominal segments, are characteristic generic features.

The present paper deals with the structures of the tracheal gill of a single species, *Palingenia longicauda* OLIV., with particular regard to the electron-microscopic structure.

Materials and Methods

May-fly larvae were collected from the Szeged reaches of the rivers Tisza and Maros. After collection the material was fixed appropriately for light and electron-microscope studies.

After Bouin, Carnoy and formalin fixation, sections either stained with haematein-eosin or impregnated with silver were prepared for light microscope histological observations. For the electron-microscope studies, excised tracheal branchial lamellae or their filaments were treated with 2% glutaraldehyde adjusted to pH 7.6 with 0.15 M phosphate buffer, and then for 2 hours with Palade fixing solution (1% OsO_4 in veronal acetate buffer (Pease, 1964), and embedded in Durcupan ACM (Araldite) resin after dehydration in an ascending alcohol series. The material was checked by examination of semi-thin sections, and the positions of the fine sections determined. The thin sections were contrasted with a lead citrate solution prepared according to REYNOLDS (1963). Photographs were prepared on Tesla BS 242 D and JEM 100 B electron-microscopes.

Abbreviations

F	=	filament	M	=	mitochondrium
Ct	=	connective tissue	Go	=	Golgi apparatus
cf	=	interstitial bundles	N	=	nucleus
tr	=	trachea	GER	=	granulated endoplasmatic reticulum
K	=	chitin	r	=	ribosome

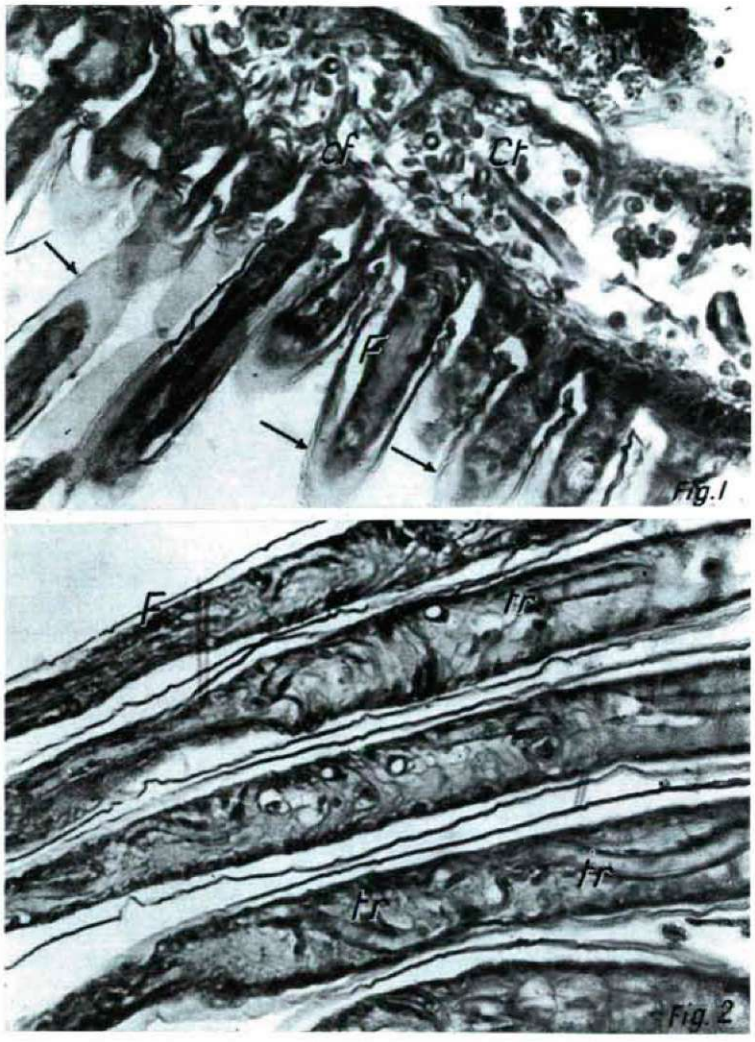


Fig. 1. Longitudinal section of the large tracheal gill.
In the lamella, interstitial cells, interstitial fibre-bundles and small tracheal branches are visible, which enter the filaments. The surface of the filaments is covered with homogeneus material (arrows). x 380

Fig. 2. Longitudinal section of filaments. Cross- and longitudinal sections of the tracheae can be seen inside the filaments. x 340

Results and discussion

Anatomy of tracheal branchia

The members of the paired branchial lamellae of the mayfly larvae are of different shapes and sizes (LANG, 1913; SCHOENEMUND, 1929; ÚJHELYI, 1959). The smaller are more rounded and nearer to the dorsal side of the abdomen than the longer, larger ones. Ventrally from them is a further densely piliated chitinous lamella, but this does not participate in the gaseous exchange.

The tracheal gill of *Palingenia longicauda* OLIV. are structured, but this structuring does not result in a symmetric arrangement on the two sides, for the filaments in the structuring are thinner on the side farther from the surface of the body and follow each other densely in a double line, in contrast with the oposite side, where the filaments are larger and arranged in a single row.

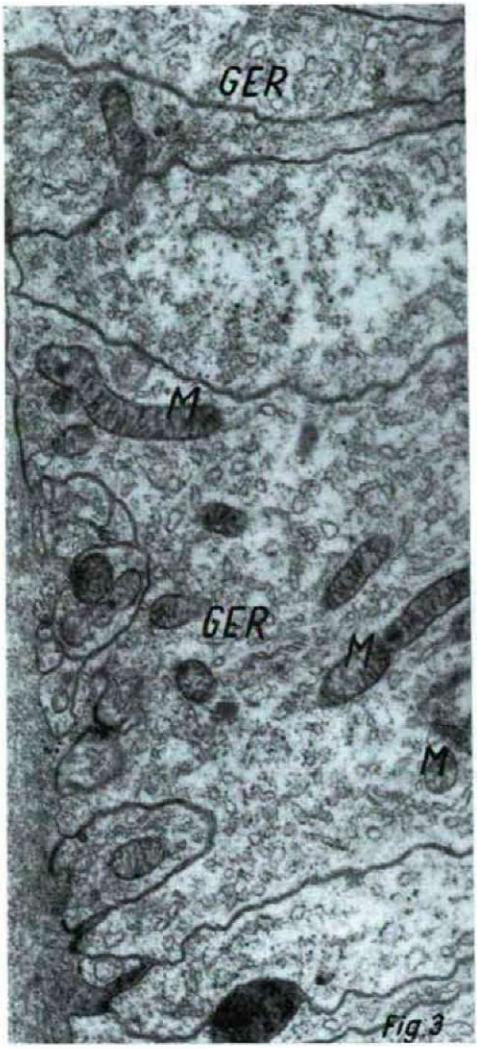
Along the entire length of the centre of every branchial lamella runs a thick tracheal branch, wich originates from the visceral tracheal trunk in the body of the larva.

This main branch divides into thinner and thinner branches, and thus the entire branchial lamellar area is covered by a network of tracheae of various diameters resulting from the gradual ramifications. The main tracheal trunk can readily be seen with the naked eye to be accompanied by a pigment layer, the thickness of which is not uniform in the region of the two lamellae. The pigment layer is thicker and denser for the larger lamella than for the smaller one. The amount of the pigment layer progressively decreases towards the tracheal gills tips, and finally disappears completely. Besides this, varying numbers of sensillae can be observed on the tracheal gills, and as described by EASTHAM (1936) for *Caenis macrura* and *Caenis horaria* they also differ in form.

Histological structure of the tracheal gill

The branchial lamellae and the filaments are covered from outside by epithelial cells arranged closely beside each other (Figs. 1–2), one of the wide bases of which becomes progressively narrower towards the inside, while at the same time the neighbouring cells appear as triangles of reversed position, since they proceed with a narrow base (Fig. 3). Such an arrangement can be particularly well seen on the epithelial cells in the region of the filaments (WICHARD et al., 1971). In addition to this, the very intense interdigitation formed with the neighbouring cells is also characteristic. The membranes of the interdigitating surfaces are fequently uniformly thickened on both sides (Fig. 5).

On the basis of their own data and also literature results on invertebrates, SATIR et al. (1970) consider the septate junction or the septate desmosome and the zonula adherens to be the characteristic forms of the epithelial junction structures. They note that there are differences, and that the tight junction too occurs for instance. This was described by LOCKE (1965) in the epidermis of insects, and by SMITH et al. (1969) in epithelial cells of the insect midgut epithel and in a certain form of the campaniform sensillae. It is striking that similar, but shorter, thickened membrane sections are found on the surface of



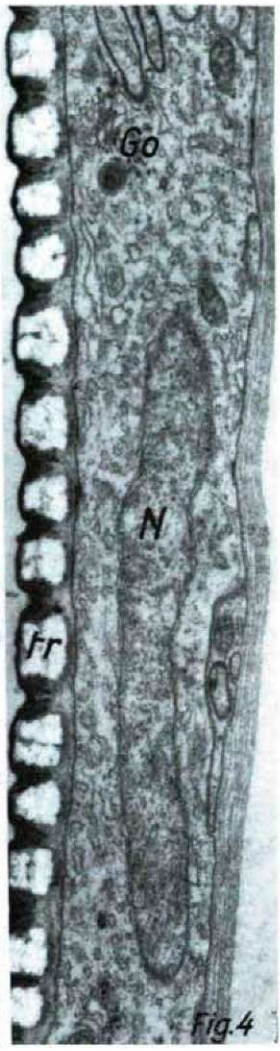


Fig. 3. Electron-microscope photograph of epithelial cells. x 19 000

Fig. 4. Longitudinal section of thin filament. Basal membrane with lamellar structure can be seen on the outside surface of the epithelial cells. Sections of the chitin rings are visible on the left side of the picture. x 12 000

the epithelial cells adjacent to the basal membrane which will be discussed below (Fig. 7). These may correspond structurally to the hemidesmosomes described by BLOOM and FAWCETT (1970). The basal membrane does not take part itself in the thickening. Desmosomes can be observed only rarely (FAWCETT, 1966) (Fig. 6).

The surface of the cells is surrounded by a homogeneous layer which can also be studied by light microscope (Fig. 1), and on this interlacing (particularly in the region of the filaments) (Figs. 1–2) or breaks (at the sensillae)

can be observed in places. The electron-microscope reveals that this homogeneous substance is composed of lamellae 80–100 Å thick, and depending on the thickness of the filaments the number of lamellae layered one on the other ranges from 2–3 to 20–25 (Fig. 4). This basal membrane also surrounds nerve bundles running on the periphery (on the outer edge of the epithelial cells) (GUPTA et al., 1969).

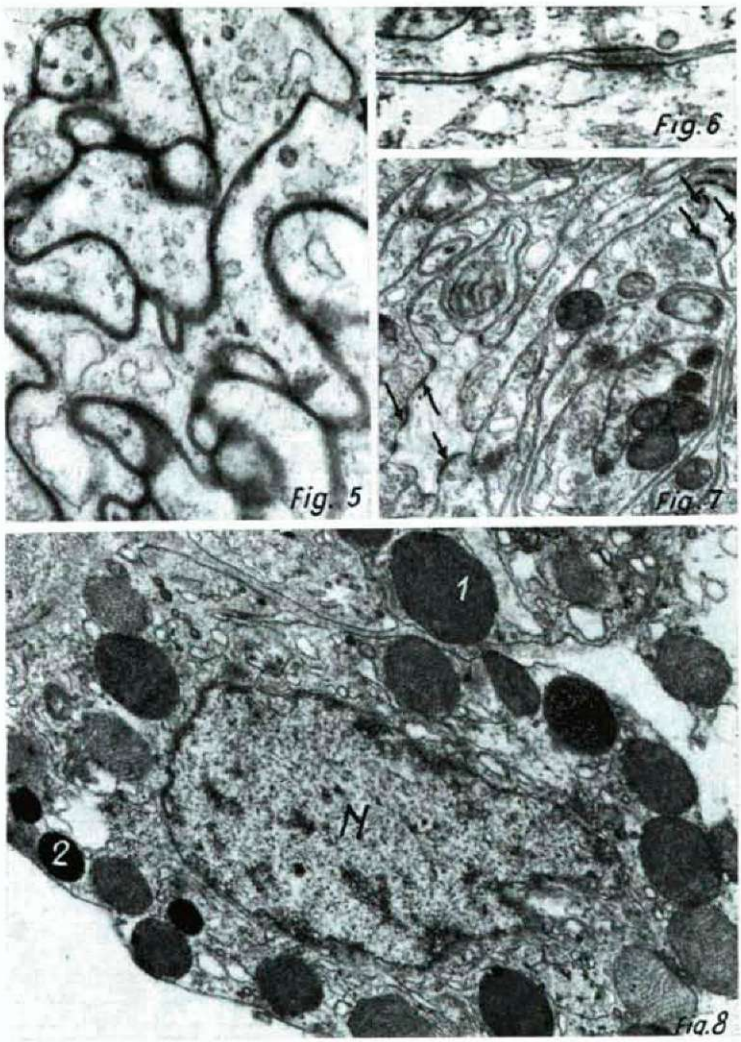


Fig. 5. Closely connected section of epithelial cells. x 31 000

Fig. 6. Desmosome between two epithelial cells. x 52 000

Fig. 7. Hemidesmosomes between the epithelial cells and the basal membrane (arrows). x 192 000

Fig. 8. Haemocytes with granules of type 1 and 2. x 16 000

The nucleus of the epithelial cells is large, and is situated approximately at the centre. A few organelles can be observed in the cytoplasm bordering the nucleus. In the entire region of the cells are found the short channels of the granulated endoplasmatic reticulum, with more rarely a parallel-running network of channels and a smooth-surfaced endoplasmic reticulum. The Golgi apparatus is slight in extent (Fig. 4), and in general dense material can be seen in the vesicles; the mitochondria are cristate type. Besides these organelles in the cytoplasm a large amount of free ribosome can also be observed. On the sections prepared from the thin filaments only epithelial cells can be seen, and between them tracheal cross- and longitudinal-sections. This structure exhibits a large degree of agreement with the structure of the branchia of *Glyphataelius pellucidus* RETZ. (Insecta, Trichoptera) (WICHARD et al., 1971).

If the histological structure of the tracheal gill itself is examined, then connective tissue can be observed below the epithelial cells covering the lower and upper surfaces; this is characteristic of an extremely loose structure, containing few cellular elements and relatively more interstitial fibres. The fibres are arranged in small bundles, some of which frequently run from the larger tracheal branches to the base of the filaments (Fig. 1).

Between the interstitial cells and fibres haemocytes are frequently to be found; these are recognizable by their typical organelles. It is characteristic of these cells that their nuclei are elliptical, while in the cytoplasm of the cell bodies of variable density can be observed, the structures of some of which can be clearly recognized whereas others appear homogeneous (Fig. 8). These special organelles can be classified according to their structure in types 1 and 2 of the haemocyte granules as systematized by SCHARRER (1972).

Summary

Anatomical and histological observations on the tracheal gill of *Palingenia longicauda* OLIV. are reported. The studies deal with the structures of the branchial lamella and the filaments. The connective structures of the epithelial cells ad their electron-microscope structures are reported.

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References

BLOOM, W. and FAWCETT, D. W. (1970): A textbook of histology. — Saunders, Philadelphia, London and Toronto.

EASTHAM, L. (1963): The sensillae and related structures on the gills of nymphs of the genus *Caenis* (Ephemeroptera). — Trans. R. Ent. Soc. Lond. *85*, 401—414.

FAWCETT, D. W. (1966): The cell. Its organelles and inclusions. — Saunders, Philadelphia and London.

GUPTA, B. L. and SMITH, D. S. (1969): Fine structural organization of the spermatheca in the cockroach, *Periplaneta americana*. — Tissue and Cell *1*, 295—324.

LANDA, V. (1948): Contributions to the anatomy of Ephemerids larvae. I. Topography and anatomy of tracheal system. — Vestnik Csl. zool. spol. sv. *12*, 25—82.

LANDA, V. (1969): Comparative anatomy of mayfly larvae (Ephemeroptera). — Acta ent. bohemoslov. *56*, 289—316.

LANG, A. (1913): Handbuch der Morphologie der Wirbellosen Tiere. — G. Fischer, Jena.

LOCKE, M. (1965): The structure of septate desmosomes. — J. Cell Biol. *25*, 166.

Palade, M. D. (1952): A study of fixation for electron microscopy. — J. Exp. Med. *95*, 285—296.

PEASE, D. C. (1964): Histological techniques for electron microscopy. — Acad. Press, New York and London.

REYNOLDS, E. S. (1963): The use of lead citrate at high pH as an electron opaque stain in electron microscopy. — J. Cell Biol. *17*, 208—211.

SATIR, P. and GILULA, B. (1970): The cell junction in a Lamellibranch gill ciliated epithelium. — J. Cell Biol. *47*, 468—487.

SCHARRER, B. (1972): Cytophysiological features of hemocytes in cockroaches. — Z. Zellforsch. *129*, 301—319.

SCHOENEMUND, E. (1929): Beiträge zur Kenntnis der Nymphe von *Palingenia longicauda* OLIV. — Zool. Anz. *80*, 106—120.

SMITH, D. S., COMPHER, K., JANNERS, M., LIPTON, G. and WITTLE, L. W. (1969): Cellular organization and ferritin uptake in the midgut epithelium of a moth *Ephestia kuehniella*. — J. Morph. *127*, 41.

ÚJHELYI, S. (1959): Ephemeroptera. — Fauna Hung. 5, 1—96.

WICHARD, W. and KOMNICK, H. (1971): Zur Feinstruktur der Tracheenkiemen von *Glyphataelius pellucidus* RETZ. (Insecta, Tricoptera). — Cytobiol. *3*, 106—110.

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ZOOBENTHOS INVESTIGATIONS IN THE SALINE WATERS OF THE GREAT HUNGARIAN PLAIN

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Introduction

The investigation of the saline waters of the Great Hungarian Plain began at the end of last century but in an organized form it has only taken place since 1961, under the auspices of the Academy Committe in Szeged, led by J. MEGYERI (PÓNYI, 1961). The research of these small waters is justified, apart from faunistic points of view, by particularly interesting physical, chemical, biological connections (KREUTZER, 1940).

The most important factors that determine their state of being and character are: their small size and periodical character. The other, biotic and abiotic, effects that influence one another in a complex way and jointly induce the fauna characteristic of the saline waters, ensue from those.

The smaller a water unit is, the more determinative the s i n g l e factors (chemical, climatic, physical, biological, etc.) become. It is not our aim to force these small water units, that are so interesting owing to their variability and plasticity, into a rigorously stereotyped form; we are rather endeavouring to recognize their fundamental regularities: all the same, some degrees of the „saline" character can be recognized on the basis of the zoobenthos investigations.

Methods

The samples were taken from three „lakes", from open water, and from near the shores, from among the vegetation, in about 200 cm^3 units. They were hand-selected after being rinsed through a metal sieve of 0.25 x 0.25 mm meshes. Then the animals were fixed in 6% formalin.

Characterization of the collecting stations

They are generally shallow waters (from a few cm to a depth of 1.5 m) of lesser extent (0.5 to 1.5 sq.km), that are in the main astatic, but in winter sometimes frozen to the bottom. The pH of the water varies between 8.2 and 10.2, according to the degree of desiccation. The quantity of water depends on the precipitation and the evaporation. Cation types: Na–Mg, anion types: CO_3–HCO_3. The shores are generally overgrown in a 1–2 m broad strip with higher-order vegetation (reed, sedge, etc.) and farther in, even some submerged vegetation may be found sporadically.

We carried out seasonal collections from 1965 until 1967 in the following saline waters: „lake" at Őszeszék and Pusztaszer – on ten occasions, „lake" at Kakasszék – on nine occasions.

As regards their origin, these saline „lakes" belong to two types (ANDÓ, 1966): those developed in an ancient river bed (e.g. Kakasszék) and those originating in deflated depressions (e.g. Őszeszék, Pusztaszer).

Lake at Őszeszék

The water is a maximum of 1,5 m deep; in the places investigated it was a minimum of 2–3 cm, and a maximum of 90 cm deep. It newer dries up completely; even in warm summers without precipitation there is a water coverage of 10–20 cm. At the shores, in a 50–150 m broad strip, there was *Phragmites–Carex* association, and in the open water *Potamogeton, Ceratophyllum* and *Chara fragilis* were to be found. The substratum is sandy clay. The water is clear, and relatively well transilluminated. The sodium content of the water is moderate compared with other saline waters. Water pollution: III.

In the material of the total of 24 collections 7616 individuals were found. The percentage of the taxonomic groups is shown by Fig. 1. The guantitative and qualitative abundances of the lake are shown unequivocally. This can be explained partly by its being less polluted, partly by its rich vegetation.

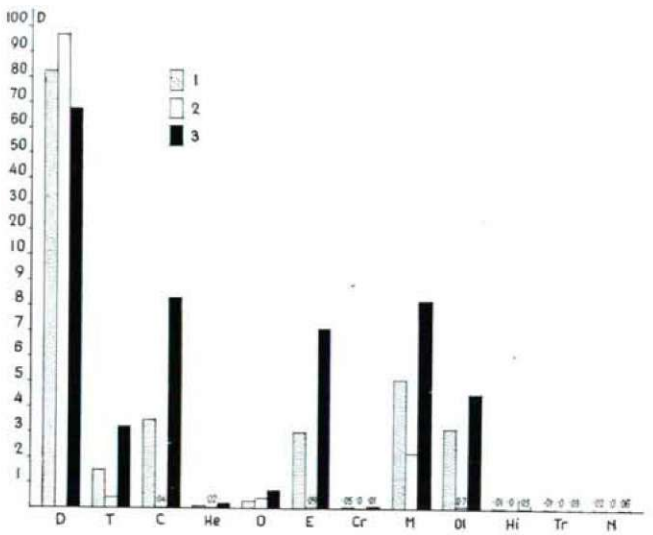

Fig. 1. Őszeszék: D values of the zoobenthos taxonomic groups

1: overall data

2: no vegetation; open water fauna

3: rich in vegetation; litoral fauna

D: Diptera, T: Trichoptera, C: Coleoptera, He: Heteroptera, O: Odonata, E: Ephemeroptera, Cr: Crustacea, M: Mollusca, Ol: Oligochaeta, Hi: Hirudinoidea, Tr: Trematoda, N: Nematoda

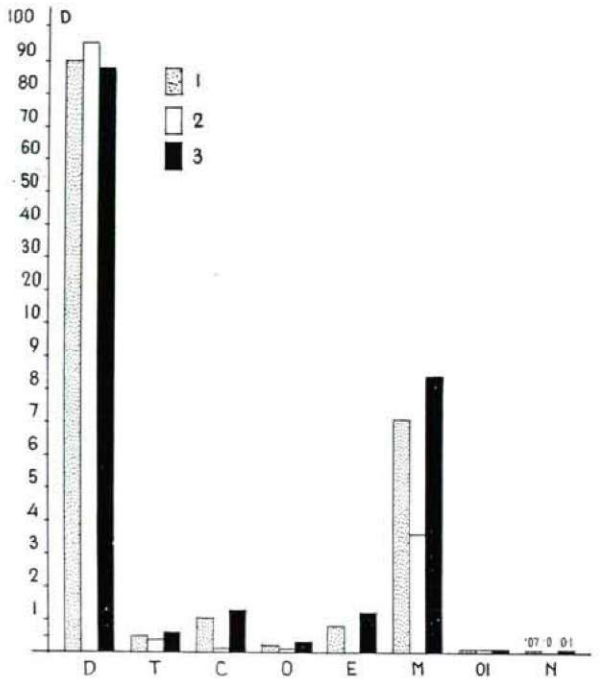

Fig. 2. Kakasszék I: D values of the zoobenthos taxonomic groups (others as in Fig. 1)

Lake at Kakasszék

Its long, narrow bed is divided by a food-path into two parts that do not communicate with each other (lake units I and II). In lake II, during the time of investigation – about half a year – a duck-farm was established, as a result of which this lake was characterized by the presence of abundant, decomposing organic matter and by sapropelic silt.

At the shore-strip of the water that dries up almost fully at intervals there are *Phragmites-Carex* associations, and only the middle of the bed is plant-free. In the parts investigated, the water depth varied between 2 and 100 cm. The substratum is sandy mud. The water is clear, limpid and comparatively well-transilluminated. The water is characterized by a very high $NaHCO_3$ content. It is extra-class IV, highly polluted.

Lake No. I: The percentage distribution of the 2769 individuals found in 28 bottom samples can be seen for the individual taxonomic groups in Fig. 2.

Lake No. II: The percentage distribution of the 3255 individuals found in 26 bottom samples can be seen for the individual taxonomic groups in Fig. 3.

The difference between the two lake units is manifested mostly in the

numerical ratio of the Oligochaeta. In lake II (as a result of the duck-manure) on the basis of the D-values of this group, this was in the second place for the four saline lakes investigated.

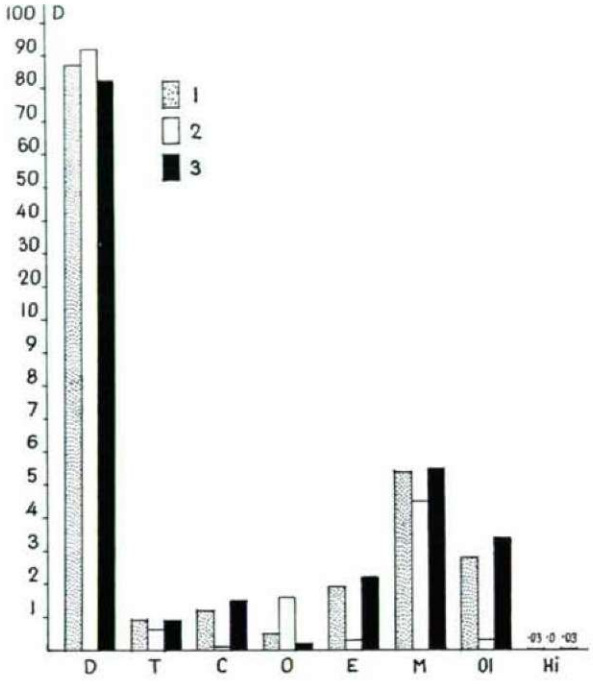

Fig. 3. Kakasszék II: Dvalues of the zoobenthos taxonomic groups (others as in Fig. 1)

Lake at Pusztaszer (Dongér)

In contrast to the above, the water of this lake is troubled, and greyish-white owing to the large mass of floating matter; it is less transilluminated. From time to time it dries up fully (summer drought). It is markedly of astatic type. It is the shallowest of the four lakes, its maximum depth being 50 cm. Its shore is sporadically dotted with a thin vegetation *(Carex, Elocharis),* with much *Nostoc* among the plants and farther in. The substratum of the open water is naked, consisting of clayey sand. It is a typcila saline water, characterized by a high soda content. It is extra-class IV, highly polluted.

In the material of the 26 collections the percentage distribution of the 5433 individuals according to taxonomic groups is shown in Fig. 4. The dominance of Diptera is the highest here; the D-values of the other taxons, however, are very low (being in this respect similar to the lake I, Kakasszék), except for Nematoda, found only here in larger numbers.

Evaluation of results

The very high D-value of Diptera agrees in all the four lake units investigated, and this in valid generally for the saline waters in this country, e.g.: Kunfehértó 78.3, Kardoskút 72.3 (FERENCZ, 1965; 1967).

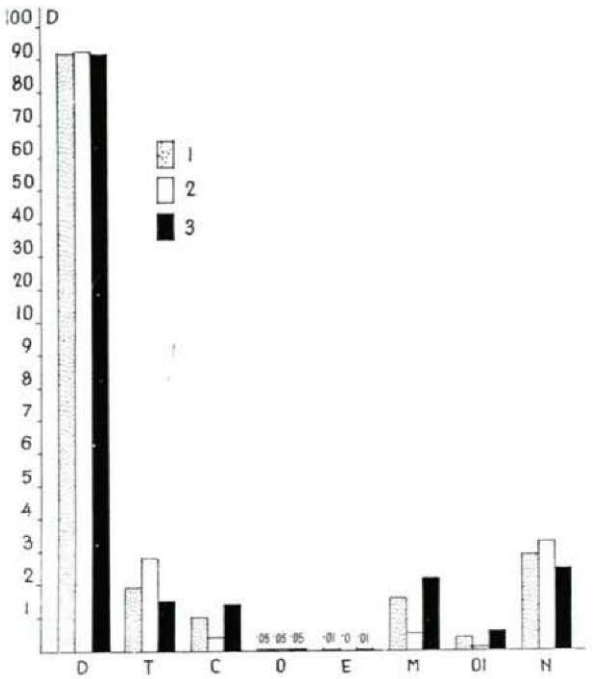

Fig. 4. Pusztaszer: D values of the zoobenthos taxonomical groups (others as in Fig. 1)

Considering the shore strip that is very rich in vegetation and the generally naked substratum of the open water (according to the fact and degree of their being stocked), we see that, on the one hand, in the fauna of the substratum in the open water the dominance of Diptera is still more marked; on the other hand, the members of more taxonomical groups live among the plants (Figs. 1–4, and Table). Where the higher-order vegetation is negligible (Pusztaszer), there is, of course, no considerable difference in the fauna of the middle water and that close to the shores.

The qualitative and quantitative seasonal change in the zoobenthos fauna is indicated by the autecology of the species. In the case of a larger water of permanent character the change in seasons makes its influence felt in only a roundabout way, but for small waters it can have a direct and fundamental effect upon the life of the species living there (e.g. if the water has dried up completely or frozen solid). Only species showing great adaptability can survive

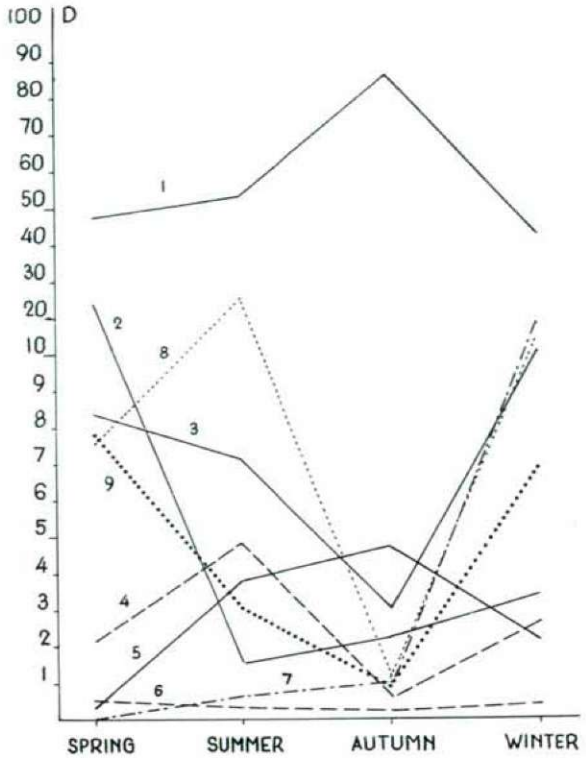

Fig. 5. Seasonal variations in the important taxonomic groups at Őszeszék
1: Chironomidae, 2: Ceratopogonidae, 3: other Diptera, 4: Trichoptera, 5: Coleoptera, 6: Odonata, 7: Ephemeroptera, 8: Mollusca, 9: Oligochaeta

under extreme conditions like these. In addition, we must consider some other abiotic factors, too, given by the shallowness and relatively small extent of the saline waters, which exert a stronger effect on their life, e.g. the great annual and daily fluctuations in temperature, the relatively larger amount of the mass of deposits as compared to the mass of water, the precipitation and evaporation, as well as the more immediate influence of the terrestrial environment, etc.

The seasonal changes in the zoobenthos populations do not show an unequivocal picture about the character of saline waters, or the water type itself. A cause of this may possibly be the unsatisfactory number of samples, and their disproportionate distribution according to the seasons, too. It must be mentioned, however, that the results of investigations of a similar character in rice-fields (KOWALSKI, 1964) were similar and that these biotops provide in many respects similar conditions for the benthos fauna, reminiscent of saline waters.

The desiccation and drying up in late summer and early autumn results in the decrease of the individual number of taxonomic groups, except for

Diptera. The prominence of the D-value of Ceratopogonidae (except in Őszeszék) in this period, when they gave for instance about 95 per cent of the total individual number in the samples got from the wet mud, is particularly striking.

The results of the zoobenthos investigations of saline waters in the Hungarian Plain therefore indicate that the major part of the species living here (Ceratopogonidae, Chironomidae) show a preference for these water types, being able to accommodate themselves in a high degree to the astatic conditions that are characteristic of these waters.

The dominant group of the saline water at Kunfehértó, that is similar to the salt lake at Őszeszék, is the Chironomidae; and in the saline water at

D-values of Diptera groups in the individual saline waters:

	Őszeszék	Kakasszék I.	Kakasszék II.	Pusztaszer
Chironomidae	85.8	78.7	78.1	59.0
Ceratopogonidae	7.7	17.1	20.0	39.5
other Diptera	6.4	4.1	1.8	1.4

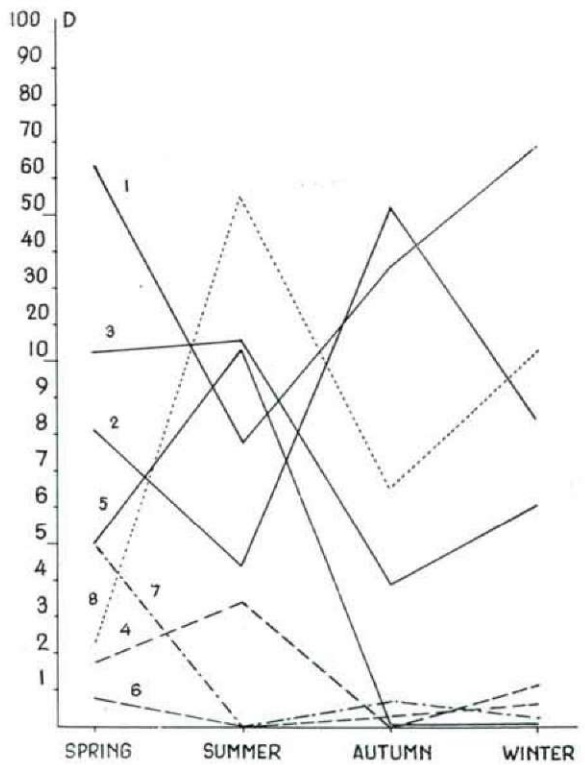

Fig. 6. Seasonal variations in the important taxonomical groups at Kakasszék I (others as in Fig. 5)

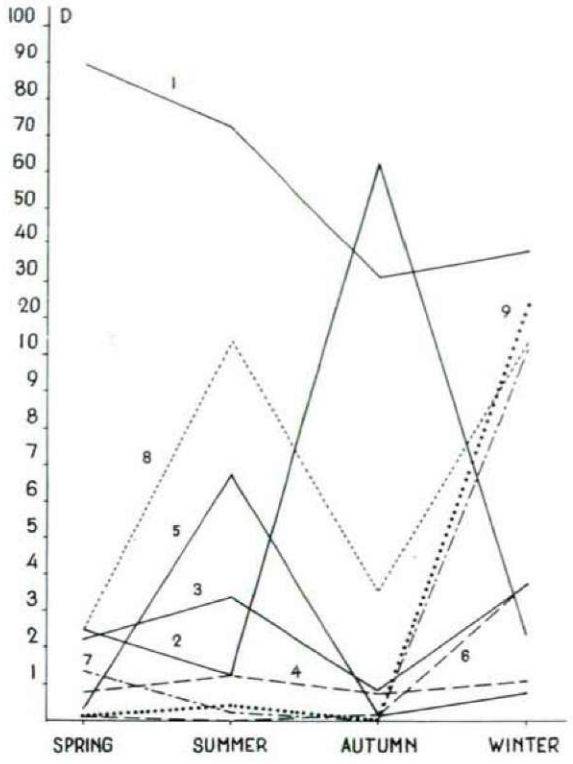

Fig. 7. Seasonal variations in the important taxonomical groups at Kakasszék II (others as in Fig. 5)

Kardoskút that, similarly to the water at Pusztaszer, has a more saline character, the dominant group is the Ceratopogonidae.

It seems that in the majority of cases the members of the groups Ceratopogonidae and Chironomidae complement one another in respect of amount. The maximum of the Ceratopogonidae is usually in autumn, in the case of decreased water and high pH (10–10,2). The maximum of the Chironomidae falls in spring (Kakasszék, Pusztaszer), or in autumn (Őszeszék).

In samples collected from below the ice, the richness in fauna of the biotops lying close to the shores and abundant in plants does not differ considerably from the others (Őszeszék). The plant-free substratum of the frozen lake at Pusztaszer, on the other hand, showed under similar conditions low quantitative and qualitative results of the zoobenthos.

The Oligochaeta, that are generally one of the most populous groups of the bottom fauna of the rivers, form only a very low percentage of the substratum fauna of saline lakes:

Kakasszék I.:	0.1	(Tubificidae:	100 %)
Kakasszék II.:	2.8	(Tubificidae:	97.87 %,
		Naididae:	2.12 %)
Pusztaszer:	0.4	(Enchytraeidae:	100 %)
Őszeszék:	3.1	(Tubificidae:	90.87 %
		Naididae:	1.24 %
		Enchytraeidae:	6.22 %
		Lumbricidae:	1.65 %)

The distribution of Oligochaeta in the three saline waters is as follows:

Őszeszék:	66.02 %
Kakasszék	26.57 %
Pusztaszer:	7.39 %

Their distribution (abundance and dominance) according to the seasons is as follows:

	A	D
spring	140	38
summer	16	4.38
autumn	46	12.60
winter	163	44.65

Taking in to consideration both the individual and the species numbers, the characteristically sodic Pusztaszer lake is conspicuous with its low values. Comparing this facts with the Ceratopogonidae group, showing a maximum D-value at the same place, the change in the ratio of the two taxonomic groups brings about the order of sequence of the saline lakes. This sequence, in the proportion of increasing sodification is: I. Őszeszék, II. Kakasszék, III. Pusztaszer.

The species of the Tubificidae familia, with the highest individual number, are distributed in the following manner:

	Őszeszék		Kakasszék	
	A	D	A	D
Limnodrilus udekemianus CLAP.	158	72.14	75	78.94
Limnodrilus claparedeanus RAT.	—	—	8	8.42
Tubifex tubifex MÜLL.	40	18.26	—	—
Psammoryctes moravicus HRABE	18	8.21	—	—
indet. Tubificidae	3	1.36	12	12.63
	219		95	

Limnodrilus udekemianus, that gave 63.83% of all the Oligochaeta, is doubtless the leading species of the saline-dwelling Annelida. Studying its autecology (KENNEDY, 1966; SZCZEPANSKI, 1953), it can be established that the limit of its tolerance towards the different kinds of water and substrata is very broad – euryvalent. According to the data of the literature and to my own observations, as well, however, the substrata of the waters that are richer in organic matter are preferred by the individuals of this species. For instance,

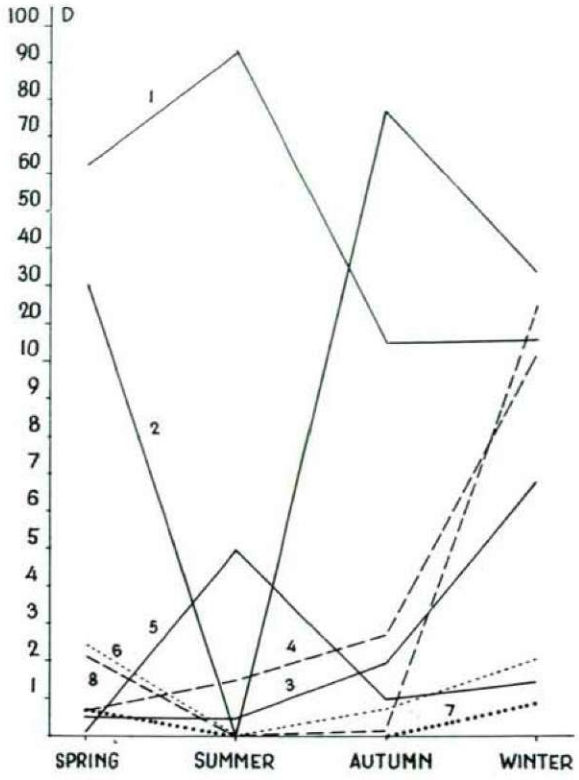

Fig. 8. Seasonal variations in the important taxonomical groups at Pusztaszer
1: Chironomidae, 2: Ceratopogonidae, 3: other Diptera, 4: Trichoptera, 5: Coleoptera, 6: Mollusca, 7: Oligochaeta, 8: Nematoda

at Szarvas (Institute of Fishculture) in the water of a shallow drainage ditch coming from a duck-pond (brought about in connection with duck breeding) they showed maximum D-values, too. It must be noted that in lake II at Kakasszék the individuals of this species were again dominant, giving about 80 % of all the Oligochaeta individulas there. And 72 % of the otherwise rich and variegated Oligochaeta population at Őszeszék belonged, to this species too. At the same place, individuals of this species were collected during the whole year, and there were even 61 individuals in a sample from the bottom of the frozen lake.

It is noted here that the individuals of *Limnodrilus udekemianus* in this water were infected in 20–25 % by a Sporozoa species: *Haplosporidium limnodrilii* GRANATA whose individuals, that were in various phases of development, frequently completely filled the cavity of the posterior body of the infected worms.

Summary

Evaluation of the results of 3 years' zoobenthos investigations in the saline „lakes" of the Great Hungarian Plain has established that in these shallow, partly astatic waters the Diptera taxonomic group is unequivocally dominant. The species of the Ceratopogonidae and Chironomidae appear generally to complement one another quantitatively. The most extreme, plantless, typical saline (natron) waters are characterized by the dominance of Ceratopogonidae, and the subdominance of Nematoda instead of Mollusca. At the same place, the Oligochaeta are represented exclusively by the Enchytraeidae family. The saline waters containing higher organic-matter pollution, and those richer in vegetation, on the other hand, are characterized by Tubificidae, and within these the *Limnodrilus udekemianus* CLAP. The seasonal changes of the zoobenthos are not characteristic and not unequivocal. The fauna of the beds rich in vegetation, however, is always more abundant.

Table

Taxonomic groups	Őszeszék		Kakasszék I.		Kakasszék II.		Pusztaszer	
	a.	b.	a.	b.	a.	b.	a.	b.
D.	+	+	+	+	+	+	+	+
T.	+	+	+	+	+	+	+	+
C.	+	+	+	+	+	+	+	+
He.	+	+						
O.	+	+	+	+	+	+	+	+
E.	+	+		+	+	+		+
Cr.		+						
M.	+	+	+	+	+	+	+	+
Ol.	+	+	+	+	+	+	+	+
Hi.		+				+		
Tr.		+						
N.		+		+			+	+

a. = open water, no vegetation; b. = litoral, with vegetation;

D. = Diptera; T. = Trichoptera; C. = Coleoptera; He. = Heteroptera; O. = Odonata; E. = Ephemeroptera; Cr = Crustacea; M = Mollusca; Ol. = Oligochaeta; Hi. = Hirudinoidea; Tr. = Trematoda; N. = Nematoda.

Species:	Őszeszék	Kakasszék	Pusztaszer
Diptera:			
Corethra plumicornis FAB.		+	
Culex hortensis FIC.	+		
Culex pipiens L.	+		
Dolichopus sp.			+
Eristalis tenax L.	+		
Stratiomyida sp.			+
Tabanida sp.			+
Tipulida sp.			+

Species:	Őszeszék	Kakasszék	Pusztaszer
Trichoptera			
Athripsodes aterrimus STEPH.	+		
Limnephilus lunatus CURT.	+	+	
Limnephilus incisus CURT.			+
Limnephilus xanthodes MCLACH.	+		+
Oecetis furva RAMB.	+		
Oecetis lacustris PICT.	+	+	+
		+	
Coleoptera			
Agabus biguttatus OLIV.	+		
Berosus spinosus STEV.	+		+
Bidessus geminus FABR.	+	+	
Coelambus parallelogrammus AHR.	+		
Colymbetes fuscus L.	+	+	
Cybister laterimarginalis DEG.	+		
Cyphon variabilis THUN.			
Dytiscus marginalis L.		+	+
Enochrus melanocephalus OL.	+		
Haliplus fulvus FABR.	+		+
Haliplus confinis STEPH.	+	-	
Hyphydrus ovatus L.			
Hybius subaeneus ERICHS.			
Laccophylus obscurus PANZ.	+		
Limnius troglodytes GYLL.	+	+	
Noterus clavicornis DEG.	+		+
Noterus crassicornis MÜLL.	+	+	
Octhebius impressus MARSH.	+		+
Peltodytes caesus DUFT.	+		
Heteroptera		+	
Corixida sp.			+
Ephemeroptera			
Cloeon dipterum L.			
Crustacea			
Asellus aquaticus L.	+		
Mollusca (+)			
Anisus spirorbis L.			+
Chondrula tridens O.F.M.	+	+	
Helicopsis striata O.F.M.		+	
Planorbis spirorbis L.		+	
Pupilla loessica LOZ.		+	
Segmentina nitida O.F.M.	+	+	
Succinea oblonga DRAP.	+		
Truncatellina cylindrica FÉR.		+	
Vallonia costata M.		+	
Vallonia enniensis GREDL.	+	+	
Vallonia pulchella M.		+	
Vertigo pygmaea DRAP.		+	
Hirudinoidea		+	
Helobdella stagnalis L.			
		+	

(+) det.: Dr. A. RICHNOVSZKY.

References

ANDÓ, M. (1966): Mikroklimaverhältnisse der sodahaltigen Teiche im sündlichen Teil der Grossen Tiefebene. — Acta Georg. *6*, 23—53.

FERENCZ, M. (1965): Beiträge zum Zoobenthos des Weissen-Teiches (Fehértó) bei Kardoskút. — Acta Biol. *11*, 265—269.

FERENCZ, M. (1967): Beiträge zur Zoobenthos-Untersuchungen des Kunfehértó. — Acta Biol. *13*, 63—70.

KENNEDY, C. R. (1966): The life history of *Limnodrilus udekemianus* CLAP. (Oligochaeta: Tubificidae). — Oikos *17*, 10—18.

KOWALSKI, H. (1964): Untersuchungen über die Bodentierwelt eines Reisefeldes. — Zschr. angew. Zool. *51*, 49—106.

KREUTZER, R. (1940): Limnologisch-ökologische Untersuchungen an holsteinischen Kleingewässern. — Arch. für Hydrobiol. *10*, 359—572.

PÓNYI, J. (1961): Zoologische Erforschung der Natrongewässer der grossen ungarischen Tiefebene. — Áll. Közl. *48*, 117—124.

SZCZEPANSKI, A. (1953): Variations of the population of the bottom living Oligochaeta in the Vistula. — Polskie Arch. Hydrob. *14*, 227—250.

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THERMOREGULATION IN THE NEST OF FORMICA PRATENSIS RETZ. (HYMENOPTERA: FORMICIDAE)

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(Received July 31, 1972)

The first data on the thermoregulative function of ant nests were published by HUBER (1820), and then on the *Formica* species, by STEINER (1926), KATO (1939), SCHERBA (1962), and KNEITZ (1969a, b). Apart from a datum of KNEITZ (1969b), no literature reference is known to the nests of *Formica pratensis*.

The investigation of the nests of *Formica pratensis* was carried out in the clearings of a lowland wood of sandy soil. The biotop is of an extreme microclimate. The thermoregulation of the nests is therefore absolutely necessary in the active season for the survival of the *Formica pratensis*, which has a comparatively limited ecological amplitude.

Methods

The temperature of the nests built of vegetal parts was measured at a depth of five cm. At the same time, the soil temperature and the air temperature at grass level were also observed. The site of investigation: the ant nests in the *Astragalo-Festucetum sulcatae* and *Festucetum vaginatae* spots of the Emlékerdő at Ásotthalom (in the vicinity of Szeged). Date: 1966 (the complete season), 1971 (July), and 1972 (from March until June). Material of the investigation: seven nests in 1966, twelve nests in 1971—72.

Results and Discussion

1. Seasonal changes in the nest temperature

The thermoregulation of the nests is manifested in a temperature which as a rule, considerably exceeds those of the soil and the air. Similarly to the results of STEINER (1926) and SCHERBA (1962), in the cold winter months was observed no thermoregulation. However, neither was observed the Winter temperature inversion described by SCHERBA (1962). In early spring, the average nest temperature relating 2 °C was 8 °C. In the course of the subsequent rise in spring temperature the difference between nest and soil temperatures increased rapidly. The higher spring temperature and the large humus content compared with that of the adjecent sandy soil result in a vegetation that is very vigorous and much higher than that of the enviroment round the nests. As a result of the shading effect of this developed plant wreath, the temperature of a large part of the mound does not exceed 30–35 °C even on warm

summer days. At the same time, the temperature of the central part, exposed to the direct radiation of the sun, may approach 50 °C (e.g. July 1966). Under such circumstances, both the workers and the sexed ants stay at the depths of the nest, or at the cooler, shady parts of the mound.

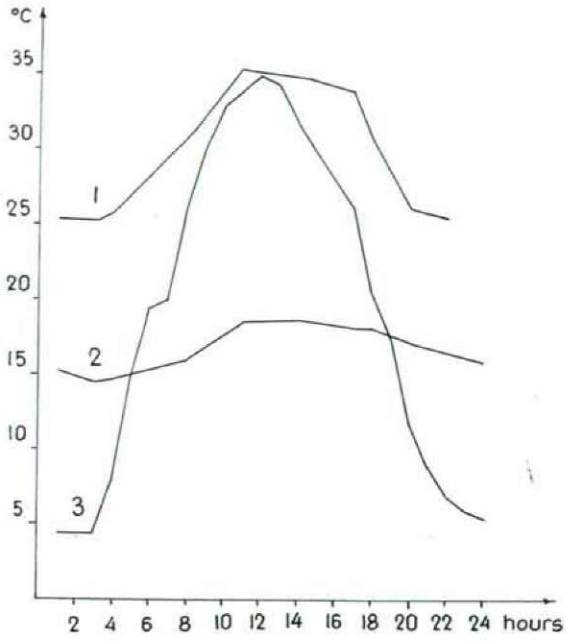

Fig. 1. Diurnal change in the temperatures of nests (1), soil (2), and air (3), 31 May 1972.

2. Diurnal changes in the nest temperature

Between 29 May and 1 June 1972 the daily changes in the temperature, its fluctuations and night minima were recorded. The temperature values observed for a clear day of adequate radiation (31 May) are given in Fig. 1. It is characteristic of the nest temperatures that they are considerable more staedy than the those of the air but more extreme than the temperature profile of the soil. On the clear, precipitation-free days of observation, the nest temperature did not fall below 25 °C and the maximum observed was 35.4 °C, meaning a fluctuation of not more than about ± 5 °C round the 28–30 °C that is optimum of the ants.

The average nest temperature, 29.75 °C is considerably higher than the daily mean temperatures of the soil (16.43 °C) and of the grass level (19.17 °C), the latter approaching the nest temperature at noon.

3. Relation between the temperatures of the nests and the environment

The thermoreguletion ensures the relative independence of the nests from the environmental factors, as well as the most appropriate milieu oecus for the ants but, at the same time, the nest temperature to a certain extent follows

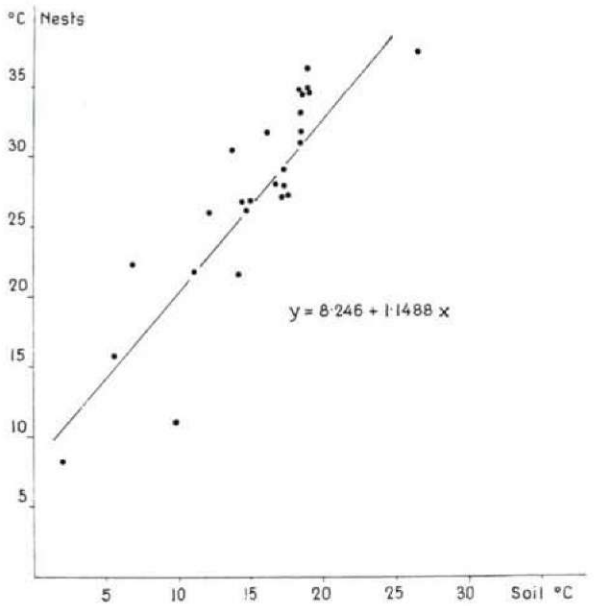

Fig. 2. Relation of the nest temperature with that of the soil.

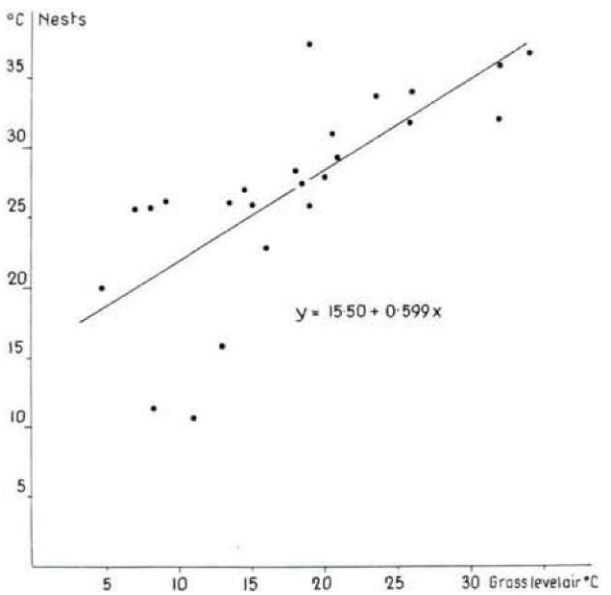

Fig. 3. Relation between the temperatures of nests and grass level.

the changes in the environmental temperature. The changes in the nest temperature are correlated to the temperatures of soil ($r = 0.6725$) and air ($r = 0.6811$) (Figs. 2 and 3).

The thermoregulative mechanism can be explained in that the insolation rays are absorved more by the surface of a loose nest made of vegetal parts than by the solid soil. This explanation is also supported by the fact that in rainy, cloudy weather the thermoregulation is smaller. In the case of Formica pratensis, the ant activity has no role in the development of a favourable temperature other than the building of the nest.

Summary

1. The temperature of the nest of *Formica pratensis* RETZ. exceeds the temperature of the environment in the active season, in this way ensuring optimum conditions for the ants.

2. Thermoregulation is ensured by a particular nest building.

3. The excessive warming up of part of the nest is impeded by the „ant-bush" developed round the nest.

References

HUBER, M. P. (1820): The natural history of ants. — London, 1—388.

KATO, M. (1939): The diurnal rhythm of temperature in the mound of an ant, *Formica truncorum truncorum* var. *yessenni* FOREL widely distributed at Mt. Hakkoda. — Sci. Rep. Tohoku Univ., Sendai *14*, 53—64.

KNEITZ, G. 1969a): Saisonale Veränderungen des Nestwärmehaushaltes bei Waldameisen in Abhängigkeit von der Konstitution und den Verhalten der Arbeiterinnen als Beispiel vortellhafter Anpassung eines Insektenstaates an das Jahreszeitenklima. — Verh. Zool. Ges. *1969*, 318—322.

KNEITZ, G. (1969b): Temperaturprofile in Waldameisennestern. — Proc. VI. Congr. IUSSI, Bern 1969. 95—100.

SCHERBA, G. (1963): Mound temperatures of the ant *Formica ulkei* EMERY. — Am. Mid. Nat. *67*, 373—385.

STEINER, A. (1926): Temperaturmessungen in den Nestern der Waldameise (*Formica rufa* var. *rufo-pratensis* FOREL) und der Wegameise (*Lasius niger* L.) während des Winters. — Mitt. Naturf. Gesell. Bern *1926*, 1—19.

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EINDRINGEN SOLITÄRER HYMENOPTEREN IN DAS NEST FREMDER ARTEN (FAM.: EUMENIDAE, SPHECIDAE, MEGACHILIDAE)

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Die Benützung verlassener, in der Regel älterer Nester durch Hymenopteren für ihre eigene Brut gehört ebenso nicht in den nachfolgend zur Besprechung gelangenden Themenkreis, wie auch der Einbruch von Parasiten in das Nest ihrer Wirte nicht behandelt wird. Gelegentlich andauernder Beobachtungen wird nämlich zuweilen die Erfahrung gemacht, dass ein Nest, das noch als bewohnt gilt, oder bloss während 1/2–2 Tage lang verlassen ist, doch durch fremde Hymenopteren in Besitz genommen wird, die dort dann kürzere oder längere Zeit hindurch ihre Tätigkeit ausüben. In solchen Fällen sprechen wir von gewaltsamer Eindringung, Raumusurpation.

Vereinzelte Beobachtungen diesbezüglich wurden durch BISCHOFF (1927), REY (1946), MICHENER (1958) und durch den Autor selbst (1961) veröffentlicht. Nach eigenen Beobachtungen (MÓCZÁR, 1961) drang *Trypoxylon figulus* L. in das noch bewohnte Nest von *Odontomerus d. deflendus* (SAUNDERS) und geriet mit dem Wirt nach dessen Rückkehr in Rauferei, schliesslich blieb aber doch *Trypoxylon* im Nest und setzte dort seine Tätigkeit fort. In derselben Abhandlung (MÓCZÁR, 1961) wurde die spezielle Verwendung der stummelhaften Flugröhre von *Paragymnomerus spiricornis* (SPINOLA) durch *Anthophora parietina* var. *fulvocinerea* DOURS mitgeteilt. In diesem Falle verscheuchte die zeitiger nistende *Anthophora* den *Paragymnomerus*, oder drang in das Nest unmittelbar nachdem sich der Wirt entfernte und baute in eigenartiger Weise auf die stummelhafte Flugröhre ihres Vorgängers ihre eigene.

Die zwischen dem 21. Juni und 16. Juli 1971 auf der tihanyer Lösswand angestellten Beobachtungen lieferten neue Angaben zur Kenntniss der Besetzung fremder Nester. Diesmal wurden mit Hilfe einer Kombination von verschiedene, farbigen Merkzeichen 109 *Paragymnomerus spiricornis* (SPINOLA) Nester und 53 tätige Wespen markiert. Ein Grossteil der Beobachtungen wurde an den günstigen Tagen (5–7 Juli, Minimum von 14–19 °C, Maximum von 35–38 °C) durch den Verfasser und seine Mithelfer dr. L. GALLÉ, Frau dr. B. HAJÁSZ und M. KÁLMÁN auf Magnetophon festgehalten. Somit konnte ein umfangreicheres Material für die Auswertung zusammengebracht werden. Hier werden nur die zwischen fremden Arten festgestellten Erscheinungen besprochen. Die Aufzählung der einzelnen Detailangaben und der Beobachtungszeitpunkte weglassend beschränken wir uns auf die Mitteilung folgender Erscheinungen.

Nest Nr. 23 von *Paragymnomerus spiricornis* (SPINOLA). Am 28. 6. 1971 fanden wir die Wespe noch in ihrem Nest. Am darauffolgenden Tag drang jedoch eine – nachträglich ebenfalls markierte – *Osmia adunca* PANZER in das Nest ein. Letztere wurde zwischen den 30. 6. und 7. 7. – mit Ausnahme von drei sehr kühlen und windigen Tagen- in 56 Fällen in dem Nest beobachtet. Am letzten Beobachtungstag verfertige *O. adunca* PANZER seine Brutkammer und verschloss sie gründlich mit einem Kotpropfen Noch am selben Tag begann *Osmia* die Flugröhre Nr. 37 von *Odontodynerus* zu befliegen. Über verschiedene Verfahrensweisen dieser Art im Nestbau berichtet übrigens auch BISCHOFF (1927 : 233). FRIESE (1923) fand sie in einem *Anthophora*-Nest. Das sonderbare befindet sich in unserem Falle darin, dass sich die *Osmia,* obzwar das Nest von dem Wirt erst kaum vor einem Tag verlassen wurde, dort bereits ansiedelte. Das Einnisten von *Osmia* kann jedenfalls auch als Raumokkupation bezeichnet werden.

Nest Nr. 37 und 108 von *P. spiricornis* (SPINOLA). Diese Wespe haben wir 33 Tage (von 21. 6. bis 23. 7. 1971) hindurch auf der Lösswand gefunden und die Tätigkeit der Wespe 10 Tage hindurch beobachtet. Im Nest 37 betätigten sich während 10 Tagen vier Wespen und eine *Osmia adunca* PANZER. Am 6. 7. und 7. 7. nachmittags betätigten sich zugleich zwei Tiere, ja am 6. 7. nachmittags befanden sich abwechselnd sogar 4 Wespen in dem Nest. Am 6. 7. nachmittags hat wahrscheinlich der Nestbauer eine gelbe Afterraupe hinausgeworfen, nachdem die Wepe einen Fremden witterte in ihrem Nest. Die im Nest gleichzeitig anwesenden Wepen wurden in Rauferei verwickelt und wollten einander vertreiben. Zuletzt entfernte sich die kleinere Wespe. Die eigentliche Wespe Nr. 37. beobachteten wir zwei Wochen später während des Baues vom Nest Nr. 108 (die in das Nest eingedrungene *Osmia* wurde bereits am Ende der vorangehend besprochenen Beobachtungen über Nest Nr. 23. erwähnt).

Nest Nr. 40 und 65 von *P. spiricornis* (SPINOLA). Diese Wespe wurde 10 Tage (von 28. 6. von 7. 7. 1971) hindurch auf der Lösswand beobachtet, ihre Tätigkeit 8 Tage hindurch registriert. Am 28. 6. besuchte *Sceliphron destillatorium* ILLIGER das durch *Paragymnomerus* bewohnte Nest. *Sceliphron destillatorium* ILLIGER trug mit den Mundwerkzeugen eine paralysierte Spinne in die Flugröhre, kam dann aus der Röhre heraus und sich am deren Rand umkehrend kroch mit dem Hinterleib voran zurück. (= Eiablage!). Nach 4 Minuten später kam *Sceliphron* zurück ohne eine Spinne in der selben Zeit wurde er jedoch durch einen *Paragymnomerus* angeflogen, der den *Sceliphron* verschenchen wollte. Die beiden Wespen gerieten in Streit, zuerst wich *Paragymnomerus* zurück, dann verliess auch *Sceliphron* die Flugröhre. Trotzdem bewohnte am 2. 7. und 4. 7. *Paragymnomerus* das Nest. Im geöffneten Nest haben wir folgendes ferfahren. Die Wespe befand sich 6 cm vom Röhrengrund entfernt mit dem Kopf nach aussen. In der Nähe des Ganges befanden sich in der ersten Brutkammer die Reste von Anthrenusfrass, in der Kammer darunter lag eine eingetrocknete Pupe eines *Paragymnomerus spiricornis* Männchens. Sie waren also Brutkammer aus dem vorhergehenden Jahr und ihre Flugröhre konnte auch eine andere gewesen sein. An dieser Stelle des Ganges habe ich ein aus Zelluloid verfertigtes Fenster angebracht, wodurch es mir ermöglicht wurde die Tätigkeit der Wespe auch am darauffolgenden Tag beobachten zu können. Nach Öffnung der sich in der Nachbarschaft befindlichen Kammer, fand ich dort 6 Neuro-


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Tafelerklärung

1. *Osmia adunca* PANZER verfertigte im Nest von *Paragymnomerus spiricornis* (SPINOLA) ihr eigenes Nest und rammt dessen Verschlussteil.
2. Teilansicht der Lösswand zu Tihany, in der Mitte mit einer kleinen Höhlung wo das *Paragymnomerus* Nest Nr. 39 und 40 war.
3. Die Brutkammer im Nest Nr. 40 nach deren Freilegung. Im Hintergrund ist das alte *Sceliphron*-Nest deutlich sichtbar.
4. Die zu den Brutkammern des Nestes Nr. 40 führende Gang nach der Verkittung. Der Insektennadel weist auf die Stelle des Zelluloidfensterchens hin.

toma-Afterraupen, am Ende des Ganges 4 Afterraupen ohne Ei. Die Wespe hatte also diese Kammer bis zum Eintreffen der kühlen Witterung noch nicht fertig gebracht. Diese Wespe verliess jedoch endgultig ihr Nest am 5., 7. und begann mit dem Bau eines neuen Nestes (Nr. 65), in dem sie sich 3 Tage hindurch betätigte, die Flugröhre baute und in vier Fällen Afterraupen brachte, und baute hier mindestens 4 Brutkammer.

Zusammenfassung

Solitäre Hymenopteren dringen auch in die Nester fremder Arten oder von solchen die anderen Familien angehören wo sie sich kürzere oder längere Zeit hindurch betätigen. Die Gründe für die Eindringung dürfen die folgenden sein: 1) Irrtum, weil diese Erscheinung auch bei solchen solitären Arten (z. B. *Osmia adunca* PANZER. Megachilidae, *Sceliphron destillatorium* ILLIG). Sphecidae) festgestellt werden konnte, die in das Nest einer gemeinschaftlich nistenden Art *(Paragymnomerus spiricornis* SPINOLA*)* eindrangen. Hinsichtlich *Sceliphron* ist der Irrtum offensichtlich, weil hinter der aufgesuchten Flugröhre sich ein *Sceliphron*-Nest befand, 2) eine gewaltsame Raumokkupation ist ebenfalls möglich die durch günstige Umstände wirksam befördert wird. Eindringung von Angehörigen derselben Art kann auch aus den oben besprochenen Gründen erfolgen, soziale Lebensweise dürfte aber öfters eine solche Erscheinung hervorrufen, die dann spezielle Folgen nach sich zieht. (MÓCZÁR, 1972). Im Falle gewaltsamer Eindringung kommt es mehrfach zu einem Kampf zwischen den Beteiligten und entweder zieht sich der schwächere Partner zurück, oder treten beide Tiere den Rückzug an und suchen eine andere entsprechende Stelle für ihre Brutkammer.

Literatur

BISCHOFF, H.: Biologie der Hymenopteren. — Biologische Studienbücher *5* (1927) 598.

FRIESE, H.: Die europäischen Bienen. — Berlin und Leipzig (1923) 456.

MICHENER, C., CROSS, E. DALY, H., RETTENMEYER, C. & WILLE, A.: Additional Techniques for Studying the Behavior of Wild Bees. — Insectes Sociaux *2* (1955) 237—246.

MÓCZÁR, L.: Gemeinsame Nester verschiedener Hymenopteren (Trypoxylon, Odynerus, Anthophora sp.). — Zool. Anz. *167* (1961) 448—455, Tab. I—III.

REY, P.: Sur le comportement d'*Odynerus spinipes* L. (Hym.) au cours de la construction de son nid. — Bull. Soc. Entom. France *51* (1946) 116—118.

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MICROCLIMATE AND THE ACTIVITY OF PARAGYMNOMERUS SPIRICORNIS (SPINOLA) (HYMENOPTERA: EUMENIDAE)

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It was found during the contiguous observation since 1939 of the wasp *Paragymnomerus spiricornis* (SPIN.) that the specimens were active preponderantly between 11 a.m. — 5 p.m., in the months June—July, on the loess wall below the Csúcshegy of the Tihany Peninsula. The intensity of their activity is closely related to the microclimate. Data concerning the vegetation of the loess wall as well as some orienting information on temperature fluctuations in front of and on the loess wall were already mentioned in earlier surveys (MÓCZÁR, 1960). To analyse more thoroughly the connection between microclimate and the activity of the wasps, opportunities arose between 21 June—16 July, 1971, when contiguous daily observations could be made (28 June—7 July) by registrating with appropriate instruments — and the help of Mrs. B. HAJÁSZ and Miss M. KÁLMÁN — the microclimatic conditions and by marking 109 wasp nests and 53 wasp individuals with the combinations of variously colored symbols (MÓCZÁR, 1972), and thus have their activity followed in minute details.

The climate of the Tihany peninsula shows warm temperate continental features. The main temperature of the warmest month is 21.5 °C, that of the coldest −1.5 °C. The peninsula belongs to that part of the Balaton Plateau where the number of clear (sunny) hours is about 1900–2000 per year.

Concerning the annual distribution of the period of sunshine, the minimum (60–70 hours) is in January, the maximum (290–300 hours) in July; its temporal distribution is closely connected with the clouded or unclouded state of the sky and not with geographical latitude. Clear conditions are encountered especially in July–August (30–40% clouded sky), a situation equal with the most sunny areas of the Great Hungarian Plain. In the areal situation of sunshine and solar energy, the physiogeographical configuration and exposition are important factors; is was established in our earlier investigations (ANDÓ, 1959) that especially the southern, southeastern, and southwestern inclinations receive a considerable amount of light and energy.

In the formation of the local climate, the immediate environment and a rich physiogeographical facies are determining features. With regard to the distribution of temperature and precipitation, the mountains are generally colder and richer in precipitation than the intramontane basins. The multiannual mean temperature value of the closed basin of the peninsula is about 10–10.5 °C, whereas that of the foothills fluctuates between 9.5–10 °C. The temporal distribution of precipitation evolves also in accordance with the typically warm continental character of the climate. Together with the early summer maximum (60–70 mm), the peninsula receives only an annual 600–650 mm of precipitation. The main winds carrying rain have a NW to W course. As to the multi-

annual average, this is the dominant wind direction in the area, though winds from the west to southwest prevail by the autumnal months. This latter phenomenon also evinces Mediterranean effects. In anticyclonal situations a local aerial circulation develops between the water surface and the dry land, a phenomenon considerably influencing the evolvement of local climate and microclimate.

The observable activity of the wasps extends to only a part of the summer period, hence we conducted our investigations in the period mentioned above and according to the following points of view:

1. The lieu and exposition of the loess wall as habitat;
2. the connections of the habitat and the solar factors:
   a. the duration of insolation of the loess wall;
3. the temperature conditions of the habitat and its environment:
   a. temperature of the loess substrate at depths of 2 and 5 cm;
   b. the changes of atmospheric temperature, maxima and minima;
   c. connections between activity observed per quarter hours and temperature;
   d. qualitative distribution of the daily activity;
   e. mean period (duration) of daily activity;
4. Aerial humidity, precipitation and wind conditions concerning the habitat its environment;
5. Summary.

1. The position and exposition of the loess wall as habitat

The nesting colony of the wasps is the steeply inclined, caved – in loess wall. The average inclination of the surface is between 20–30°. In certain cases (as also in that of the habitat), very steep declivities, up to a value of 90 ° or even more, originated by tectonic movements. The surface erosion of the loess wall is not significant owing to its protection againts wind by the considerable forest stands of the immediate neighbourhood. The exposition of the habitat slope was in all cases southern to soutwestern: on western slopes few and on nortwestern ones no nests were to be found. This is explainable by the considerably different light-climate exposition of the slopes, of which the S–SW exposition received the greatest amount of insolation. In the case of a 20 °C declivity for instance, light energy and the angle of inclination of sunshine are twice as much on a southern to southwestern slope at noon during the summer than on a northern slope (ANDÓ, 1959).

The environment of the S–SW loess wall inhabited by the wasps consists, phytocoenologically, of steppe swards and karst shrubs (*Festucetum sulcatae* and *Cotino-Quercetum*); it is owing to this fact (MÓCZÁR, 1960) that the wasp settled and breeds on the loess wall, as reported several times in the past 34 years (MÓCZÁR, 1939–1972). In Hungary, the wasp nests in one other place only, the corresponding slopes of the Mts. Villány, South Hungary; otherwise the species ranges in the Mediterranean region.

2. Connections of the habitat and solar factors

a. The duration of insolation of the loess wall

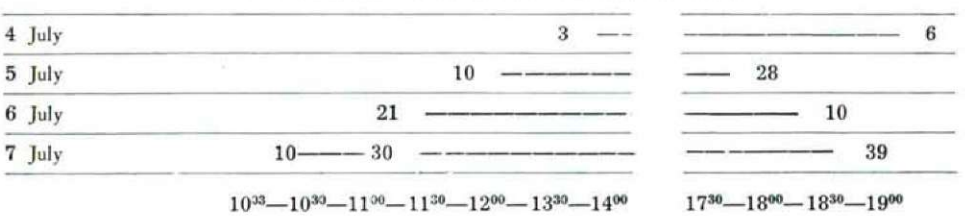
The southern and southwestern exposition as well as the angle of inclination of the loess wall assure the obtaining of a considerable amount of light and energy. The hourly sequence of magnitude of insolation angle and energy values for the horizontal level (calculations in Central European Mean time), in the period 26 June–8 July, 1971, was at Tihany as follows:

Table 1. Insolation and energy values per hour

Hour	Insolation angle values	Cal/cm²/min¹
4 h 48″	7° 3′	0.0368
5 h 48″	16° 35′	0.1855
6 h 48″	26° 37″	0.4001
7 h 48″	36° 51′	0.6297
8 h 48″	46° 53′	0.8161
9 h 48″	56° 5′	0.9958
10 h 48″	63° 13′	1.116
11 h 48″	66° 5′	1.156
12 h 48″	63° 13′	1.116
13 h 48″	56° 5′	0.9958
14 h 48″	46° 53′	0.8161
15 h 48″	36° 51′	0.6297
16 h 48″	26° 37′	0.4001
17 h 48″	16° 35′	0.1855
18 h 48″	7° 3′	0.0368

On the loess wall, values double owing to the angle of inclination and exposition. The amount of sunshine falling on the wall is especially high between 11 a.m.–5 p.m. This is the main reason why the activity of the wasps starts relatively late in the forenoon, and continues in essence in the afternoon only so far and only in sites where the wall is insolated. In sunny, clear weather the wasps are active also between 10 a.m.–7 p.m. The earliest and latest activities are illustrated in Table 2.

Table 2. Number of earliest and latest active wasps.



It is worthy of note that, according to TSUNEKI (1969), *Odynerus frauenfeldi* SAUSSURE flies out as the earliest between 7^{01}–7^{30} and at the latest between 15^{00}–16^{30}, being active occasionally until 17^{00}–17^{30}.

3. Temperature conditions of the habitat and its environment

a. Temperature of the loess substrate at depths of 2 and 5 cm

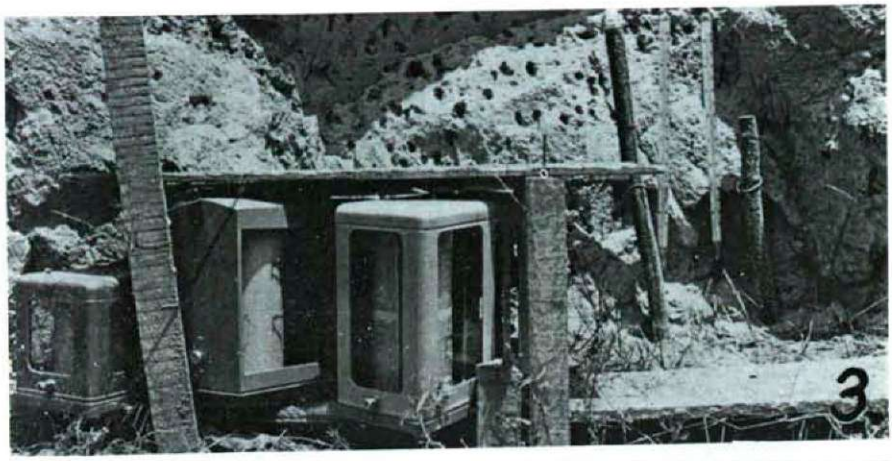
On clear and sunny days (5–6 July), the warming-up of the loess wall presented a peculiar picture. The rate of warming-up is illustrated by the graphs of Figs. 6–8. It is rather conspicuous that the surface and the air layer immediately above it heat up rapidly, while the heat diffuses rather slowly

Fig. 1. A part of the western slope of the loess wall at Tihany.
Fig. 2. The cup anemometer and the Assmann psychrometer in front of the Paragymnomerus nests.

within the loess substrate. Two cm below the surface, the magnitude value of the temperature during the forenoon warming-up phase lags, as related to that of the air 10 cm above the surface, 2 hours, and at a depth of five cm 4 hours. An isoplete illustration (Fig. 9) displays the distribution of warming-up, with reference to the amount of heat received and the heat regime, of the surface, the atmosphere and the loess substrate. The immediate surface layer to a depth of 0.5 cm can be considered a definitely warm layer, the same as the air layer directly above it.

The value of the correlation coefficient between the temperature values observed at a depth of 2 cm and the activity of the wasps is still positive and comparatively great (0.8608 ± 0.0722). A close stochastic relationship exists

Fig. 3. Temperature recording instruments (barograph, termohygrograph, psychrograph), with the soil thermometers at 2 and 5 cm depths (right side).
Fig. 4. Minimum thermometer on the slope near the loess wall.
Fig. 5. Hygrograph (left), soil thermometer and thermograph placed at the Balaton level.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between the two variables. In the investigated temperature and activity interval, the approaching regression function of the connection is linear (Fig. 10).

The value of the correlational coefficient between the temperature values measured at a depth of 5 cm and the activity of the wasps is low ($0.2444 \pm \pm 0.297$), hence it cannot be brought into relationship with the activity of the wasps on the surface of the loess wall (Fig. 11). Temperature changes within the wall influence rather the development of the larvae reposing in the excavated brood chambers.

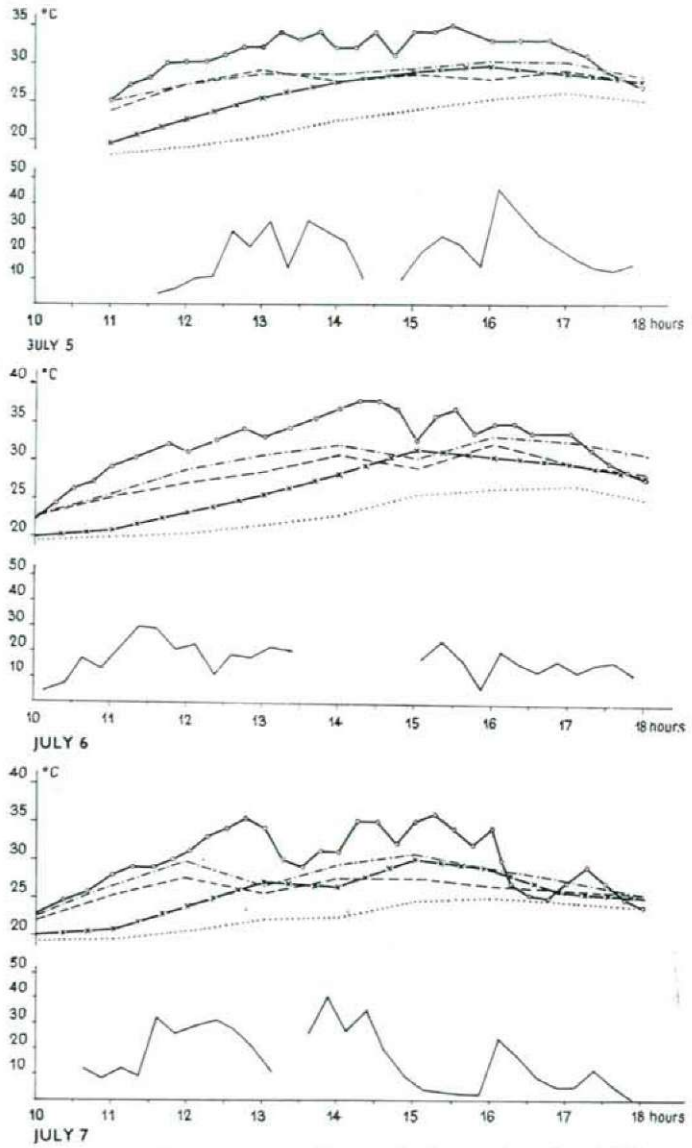

Fig. 6—8. Temperature recorded per quarter hour and the number of activities on 5, 6, 7 July.

b. Changes of atmospheric temperature, minima and maxima

In the investigated period, atmospheric temperature was not invariably favourable, but rather separable into three macrosynoptic situations:

cloudy, rather warm weather with showers,
variably overcast sky, very cold weather,
wind-free, clear, warm weather.

This latter was experienced between 5–7 July and on some subsequent days; prior to this, the prevailing weather was extraordinarily cold (1–2 July). The daily temperature mean fluctuated between 13–15°C, less by 5–7 °C than the mean calculated for the previous hundred years. In clear, warm, dry, windless weather the daily temperature mean exceeded 20 °C, indeed, it was + 1.3 °C higher on 6 July than again the daily mean calculated for hundred years.

The daily temperature data of the loess wall and its environment are given in Figs. 6–8. The changes of the atmospheric temperature were composed from a series of data. The termograph was continuously operating near the nesting site of the wasps on the loess wall (Fig. 3, left). We took readings every hour by an Asmann psychrometer, first on the site of the loess wall preferred by the Sceliphrons, then in a cavity then at a height of 1 m immediately in front of the wall (Fig. 2). Of these, the first and the third data gave more reliable data in view of the nests of the wasps. The termograph ran invariably in the same place, therefore it gave even more reliable data than the values measured by the manual instrument read every hour.

The minimum and maximum values measured on the days under discussion are submitted in Fig. 12. on 5 July, we measured the 35 °C maximum at 15^{30} (Fig. 6), but the maximum activity was between 16^{00}–16^{15}. The 36 °C maximum on 6 July was at 14^{30} (Fig. 7), while the maximum activity fell between 13^{45}–14^{00}. It should be added that on the same day we measured 35 °C at 12^{45}, on the basis of a continuous warming-up, therefore a maximum temperature was present already then. After an also uninterrupted warming-up, on 7 July, the maximum 38 °C was read at 14^{30} (Fig. 8), while the maximum activity occurred between 11^{15}–11^{30} (the possible source of error might lie in the fact that we observed only a part of the loess wall between 14^{00}–15^{00}).

It appears therefore that the maximum activity follows the onset of the temperature maximum.

c. Activity observed per quarter hours and temperature

For the evaluation of the relationship between activity and atmospheric temperature, the days 5–7 July were the most suitable. Activity numbers recorded at the points of time of observation and the corresponding temperature values per quarter hour are given in detail in Figs. 6–8. Owing to the causes given above, we record here the thermograph data.

On 5 July, temperature started with 25 °C at 11^{00}, reaching after a largely gradual increase 34 °C by 13^{15}. Also activity increased gradually, from 4 to 33 per quarter hour, falling back to 23 only between 12^{45}–13^{00} – in a period

when temperature remained unchanged (31.9 °C) but when the sun was obstruction by a cloud and to this the wasps reacted sensitively. When temperature fell by 1 °C (33 °C) at 13^{30}, also activity dropped consideably (to 15). This phenomenon repeated itself between 13^{45}–14^{30}. At 14^{45} temperature fell by 3 °C (to 31 °C), with a corresponding decrease also in activity. Between 14^{45}–15^{30} temperature again reached by 4 °C the daily maximum (35 °C), so that the number of active wasps increased again, therefore after the smaller relapse owing to the clouded sky activity maximum again rose to 46 in the wake of the temperature maximum. After 16^{00}–16^{14} both temperature and activity decreased gradually together.

On 6 July, temperature was 23 °C at 10^{00}, with the maximum 35 °C reached at 12^{45}. Activity, with some smaller fluctuations, reached its first maxima (32 and 31, respectively) between 11^{30}–11^{45} and 12^{15}–12^{30}. Subsequently, temperature fell 6 °C till 14^{15} (29 °C), markedly observable also on the activity of the wasps. Besides the two smaller drops in temperature (3 °C on both occasions), a greater decrease (10 °C, resulting in 25 °C) happened only at 16^{45}; before the evening cold, this was followed yet by a smaller warming up period (29 °C at 17^{15}). Owing to these latter fluctuations in temperature, activity

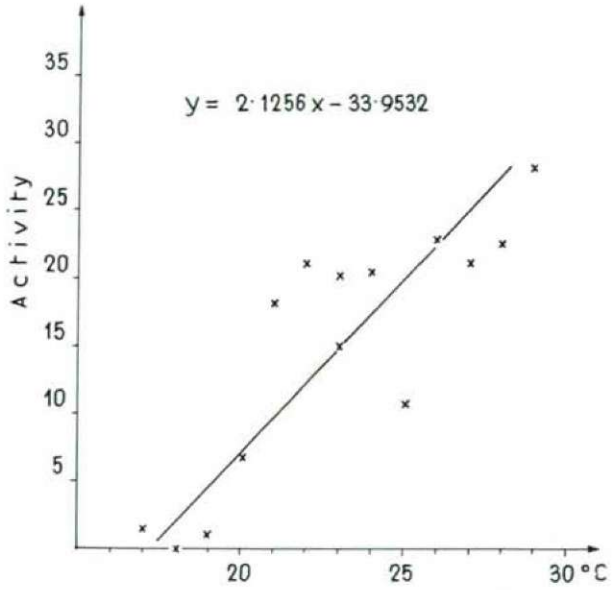

Fig. 10. Regression of connection between temperature of loess wall measured at a depth of 2 cm and the activity of the wasps.

also seemed to decrease considerably, and the number of active wasps increased significantly only after the temperature maximum mentioned above.

On 7 July, temperature rose from 22 °C at 10^{00} to 38 °C by 14^{30}, therefore by 16 °C. Subsequently, it fell back considerably at 15^{00} and 15^{45}, decreasing gradually to 28 °C by 18^{00}. The course of activity is less unequivocal. Most active wasps (30) were observed between 11^{15}–11^{30}, and though temperature continued to rise – even if occasionally dropping somewhat owing to the

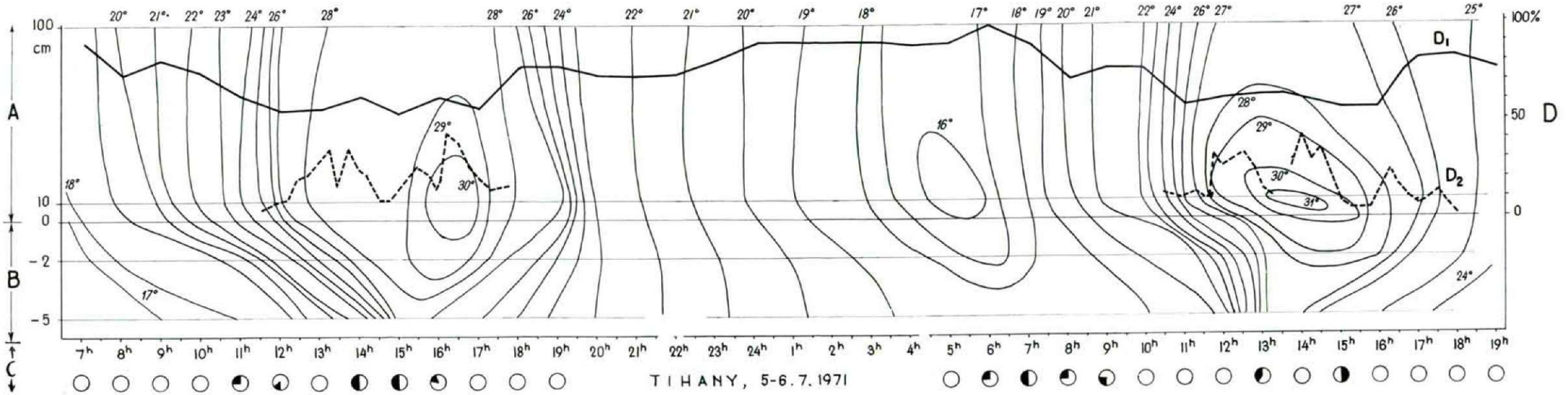

Fig. 9. Temperature distribution in the isoplete system. A = atmospheric temperature; B = soil temperature; C = values in hours of sunshine duration and temporal line; D = per cent scale; D/1 = atmospheric humidity in per cent; D/2 = activity in per cent.

passing of clouds – it failed to increase activity. The falls in temperature at 15^{00} and 15^{45} had significantly decreased also activity.

By a statistical analysis of the atmospheric temperature and the activity of the wasps it can be established that the correlational connection of activity is the closest with the temperature measured by the thermograph ($c = 0.986 \pm \pm 0.0065$). This result completely agrees also with the data of Figs. 6–8. The regressional connexion of atmospheric temperature and activity is linear in the investigated interval, similarly to that of the temperature of the loess wall measured at a depth of 2 cm (Fig. 13). A less close stochastic relationship could be observed with the temperature measured by the Assmann psychrometer at a height of 1 m ($c = 0.9119 \pm 0.0415$) or in the cavity of the loess wall ($c = 0.719 \pm 0.145$). The cause lies in all probability in the measuring technique discussed above. Our newest, 1972 observations seem to substantiate the assumption that the activity of the wasps still increases to a temperature about 40 °C, reaching its maximum there, and then it conspicuously falls back.

d. The qualitative distribution of the daily activity

Of the various activities of the wasp, a prolonged observations is mainly possible of the building of the nest (that is, the digging of the pupal chamber, the construction of the turret or the discarding of the excavated earthen pills) and the transportation of the sawfly larvae. According to our observations, the wasps were busy constructing, between 10 a.m.–6 p.m., in the following number (= amount, and not numbered!) of turrets or carrying sawfly larvae for food (the amount of this latter given in brackets):

Table 3. Qualitative distribution of daily activity. Numbers refer to the amount of nests under construction, those in brackets designating the total of nests into which sawfly larvae were transported.

5 July	—	—	7	20	21	23	24	14	4
	—	—	(2)	(6)	(4)	(3)	(4)	(7)	(1)
6 July	—	13	9	22	24	22	15	5	1
	—	(3)	(6)	(2)	(9)	(2)	(7)	(2)	(1)
7 July	1	15	16	20	21	22	21	11	—
	(4)	(6)	(12)	(6)	(4)	(2)	(5)	(8)	(3)
Total	1	28	32	64	66	67	60	30	5
	(4)	(9)	(20)	(14)	(17)	(7)	(16)	(17)	(5)
	10	11	12	13	14	15	16	18	hours

Accordingly, most wasps were constructing nests between 12^{00}–16^{00} hours, and most were carrying sawfly larvae between 11^{00}–14^{00} and 15^{00}–17^{00} hours.

If the numbers of constructing or larva-transporting wasps are compared with the daily mean temperature data (Figs. 6–8), we find the following picture:

The greatest number of wasps were constructing their nests on 5 July in the hours of the temperature maximum (14^{00}–16^{00}); on 6 July in the hours between the temperature maxima (12^{00}–15^{00}); on 7 July in the hours of the temperature maximum (14^{00}–15^{00}) and in the two hours preceding it (12^{00}–14^{00}). As far as the transportation of sawfly larvae for food was concerned, the wasps

were carrying the larvae also during the hour after the maximum (15^{00}–16^{00}) on 5 July; in the hour following the first or the forenoon maximum (13^{00}–14^{00}) (at the time when temperature had conspicuously decreased) on 6 July; and in the forenoon hour (11^{00}–12^{00}) of the still warming up temperature, as well as following the maximum (16^{00}–17^{00}) on 7 July.

e. Mean period (duration) of daily activity

If observations running merely to one or two days are disregarded and only those extending over at least 3 days are taken into account, the following periods, with respect to the daily activity of the individual wasps, are obtained (Table 4):

Table 4. Period totals of the daily activity.

Serial number of wasp	Daily activity/hour on July					Daily mean of work
	3rd	4th	5th	6th	7th	
15			4.50	2.37	6.29	4.47
18	0.32		5.34	4.25	6.31	4.16
19	0.52	2.58	5.31	6.36	5.09	4.12
23	4.57		0.17	4.42	2.37	3.00
28	3.38	3.39	0.57	6.49	7.38	5.43
29			5.19	5.44	6.32	5.52
30		4.15	5.47	6.22	5.19	5.26
31		3.11		2.06	6.34	4.33
33			6.00	4.52	5.04	5.19
34			4.28	3.25	1.57	3.17
35	0.44		2.03	5.59	5.00	3.29
36			2.22	6.40	7.39	5.34
37			4.33	2.40	5.02	4.05
38			5.08	6.40	6.04	5.58
39	6.15	4.46	2.04	3.53	4.55	4.46
46	1.19	0.40		5.08	6.20	3.22
47		5.02	1.53	4.37	6.08	4.25
48			4.50	6.53	4.45	6.09
50			4.00	5.18	5.03	4.47
56			4.24	0.04	2.55	2.27
57		3.57	2.25	6.54	6.20	4.54
59		0.13	5.45	4.46	0.31	2.48
61			4.58	5.34	7.11	5.54
64			4.57	4.03	7.38	5.32
65			5.22	7.16	7.31	6.42
66			4.24	7.18	6.17	5.59
68			4.16	5.17	0.04	3.07
72			4.27	2.45	6.19	4.30
75			2.57	8.24	5.28	3.48
81			1.16	6.28	4.11	3.56
85			5.12	3.01	0.31	2.55
86			2.08	6.06	1.55	3.23
87			2.11	6.42	7.22	3.15
89			3.10	1.01	5.49	3.20
90			0.08	1.56	2.02	1.22
93			1.27	5.11	6.42	4.26
94			0.49	5.15	7.01	4.22
102				4,34	4.03	4.21
						158.01

According to the above data, the daily activity of the wasps fluctuate between rather wide limits. Even on the day of the most favourable temperature (7 July), Wasp No. 68 spent no more than 4 minutes in its nest, whereas Wasp No. 36 was active for 7 hours and 39 minutes on the loess wall. The totalling of the data shows that during the above observation days the 38 wasps were active for an average of 4.158 hours on the surface of the loess wall held under observation.

4. Atmospheric humidity, precipitation and wind conditions of the habitat and its environment

The per cent distribution of the annual wind direction in the region discussed is as follows:

NW	18%	S	5%
N	10%	SW	12%
NE	8%	W	19%
E	12%	Wind free	10%
SE	6%		

The area is on the lee side, but the vegetation gives complete shelter against winds for the nesting site of the wasps. Though aerial movements measurable with an anemometer (Fig. 2) could be observed in the case of a stormy wind force, even this movement was so slight at the loess wall that

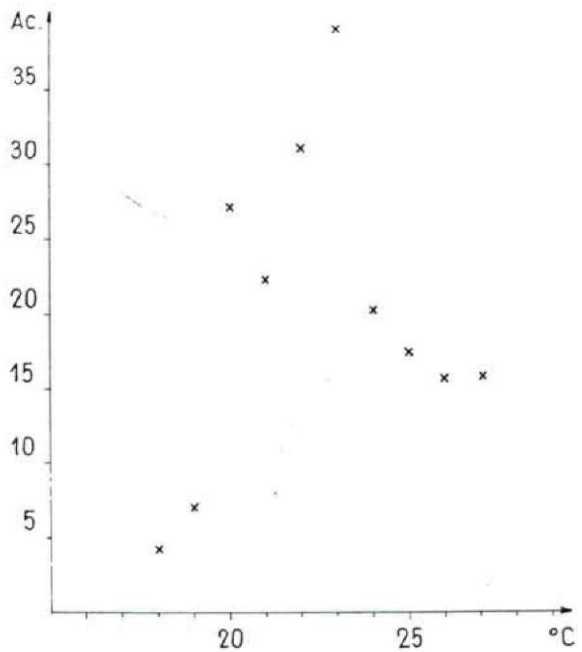

Fig. 11. Connection between the temperature of the loess wall measured at a depth of 5 cm and the activity of the wasps.

it had not influenced the activity of the wasps in the least. Atmospheric humidity conditions, on the other hand, affected the wasp to a considerably greater extent.

Paragymnomerus spiricornis has a Mediterranean range, and on the basis of its stenök eremophilous character one may presume that an increase in the

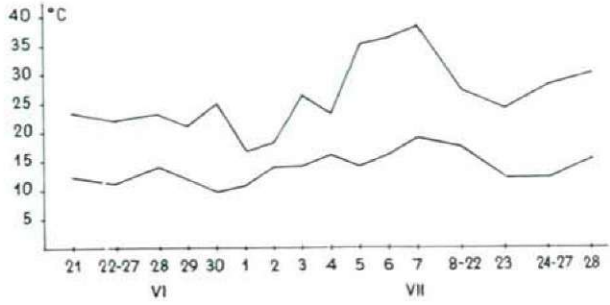

Fig. 12. Minimum—maximum data of the loess wall and its environment.

atmospheric humidity will influence the activity of the wasps rather to disadvantage. Instrumentated observations in this year have shown that the activity of the wasps is inversely proportional with the relative humidity content, and this was corroborated also by the correlational analysis ($C = -0.9728 \pm \pm 0.0112$).

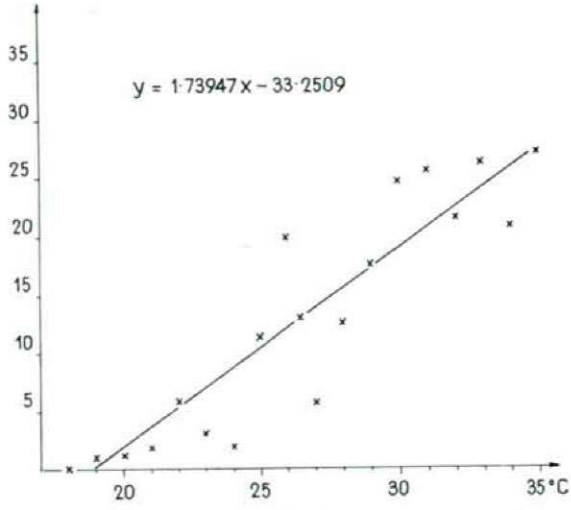

Fig. 13. Regression connection between atmospheric temperature in front of the loess wall and the activity of the wasps.

Since atmospheric humidity during the daytime is higher in the forenoon hours than in the afternoon, also this factor – in close connection with temperature – must have considerably influenced the activity of the wasps.

5. Summary

The Tihany Peninsula has a warm climate of a continental character. It receives as much sunshine as the most insolated part of the Great Hungarian Plains. The region has most characteristic local and microclimate conditions; especially the exposed hillsides show climatic features which determine the several life communities. Thus the position of the loess wall and the *Festucetum sulcatae* and *Cotino-Quercetum* environment afford nearly uniquely suitable conditions for the gregarious colonization of the site by *Paragymnomerus spiricornis* (SPINOLA). The exposition of the loess wall and the sheltering effect of the surrounding vegetation render the site free of aerial movements, a slight breeze occurring there only in stormy and tempestuous weather in the neighbourhood. The SW exposition of the slope, inclined nearly 90°, is directly insolated from about 10–11 a.m., so that, owing to the steep inclination, the considerable amount of energy causes a rapid upward increase of temperature, inciting – between 11 a.m. and 7 p.m. – the activity of the wasps.

The value of the correlational coefficient between the subsurface temperature of the wall (measured at a depth of 2 cm) and the activity of the wasps is positive and great, the regressional function of the relationship is linear. Temperature values measured at a depth of 5 cm influence rather the development of the larvae in the pupal chambers. Aerial humidity content is inversely proportionate with the activity of the wasps, the value of the correlational coefficient is -0.9728 ± 0.112. The activity of the wasps is mostly influenced by the atmospheric temperature in front of the loess wall (the correlational coefficient is 0.986 ± 0.0065). Maximum activity sets in after the temperature maxima. Activity figures, totalled per quarter hour, correspondingly followed the changes in temperature observed by a thermograph on 5 and 6 July. On 7 July, the two failed to exhibit this close connexion, though still in agreement. Drops in temperature or even the passing of a larger cloud causes a decrease in activity. Concerning the qualitative distribution of activity, the greatest number of wasps was observably occupied in constructing nests between 12^{00}–16^{00} in the suitable warmth and carrying sawfly larves for food between 11^{00}–14^{00} and 15^{00}–17^{00}. Nest constructing occurred mainly during the hours of the temperature maximum, while the transportation of the sawfly larvae in the preceding or the subsequent hours. The period of daily activity of the wasps fluctuates between 4 minutes and 7 hours 39 minutes. Thirty-eight numbered (marked) wasps were active on the loess wall for a mean period of 4 hours and 15 minutes per individual during 5 days of observation.

References

ANDÓ M. (1959): A Tihanyi-félsziget mikroklimatikus viszonyai. (Handscript).

MÓCZÁR L. (1939): Beobachtungen über den Nestbau einiger Odynerus-Arten. — Zool. Anz. 125: 69—80 Abb. 1—24.

MÓCZÁR L. (1960): The Loess Wall of Tihany and the Nesting of Odynerus spiricornis Spin. (Hym Eum.). — Ann. hist. — nat. Mus. Nat. Hung. 52: 383—409, Pl. I—V.

MÓCZÁR, L. (1974): The Activity Periods of the Population of *Paragymnomerus spiricornis* (Spinola) (Hymenoptera). — Acta Biol. Szeged. (in print).

MÓCZÁR, L. (1974): The Unusual Behaviour of *Paragymnomerus spiricornis* (Spinola((Hymenoptera Fam.: Eumenidae). — Acta Biol. Szeged. (in print).

TSUNEKI K. (1969): The Nesting Activity of Odynerus frauenfeldi Saussure. — The Life Study (Fukui) 13 (1—2): 1—12.

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DATA TO THE ELECTRON MICROSCOPIC STRUCTURE OF THE PINEAL ORGAN OF THE BIRDS

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Of late, the results of several electron microscopic investigations in respect of the pineal organ of the bird were published (BISCHOFF, 1967; 1969; BISCHOFF and RICHTER, 1966; COLLIN, 1966; 1967; 1968; FUJIE, 1968; MIKAMI, 1968; 1969; OKSCHE, 1965; 1968; OKSCHE–KIRSCHENSTEIN, 1969; OKSCHE and VAUPEL von HARNACK, 1965; 1966; RENSONI, EAKIN and QUAY, 1968; QUAY, 1965; 1968; UECK, 1969). The statements concerning the structure were completed and put in a new light by experimental, extirpation-, electrophysiological, bio- and histochemical investigations carried out similarly on different birds (AXELROD, WURTMAN, 1966; AXELROD, WURTMAN and WINGET, 1964; ARIENS KAPPERS, 1965; KELLY, 1962; KOBAYASHI, 1969; MORITA, 1966; MENAKER, 1965; 1968; 1969; MENAKER and KETT, 1967; OKSCHE, MORITA and VAUPEL von HARNACK, 1965; 1966; RALPH and DAWSON, 1968; QUAY, 1966; QUAY and RENZONI, 1963; WURTMAN, 1969; WURTMAN, AXELROD and FISCHER, 1964; WURTMAN, AXELROD and KELLY, 1968).

Comparing the different results obtained from the pineal organ of the various bird species to the large number of data found in sub- and superavian vertebrate groups respectively in the human (BARGMANN, 1943; DODT, UECK and OKSCHE, 1971; KAPPERS, 1971), we may establish that there isn't in the organism another organ as unknown as this peculiar protrusion of the thalamic tegmen.

In spite of the multilateral investigations and multisense illuminations, it can only be said even to-day that there is no certainty as to the origin and quality of its cells (neural, glial, ependymal, parenchymal), resp. the function of the whole organ (sense organ, receptor, photoreceptor, neurohumoral or exocrine, resp. endocrine gland or a rudimentary remain).

As not only the different classes of Vertebrate but even within these, in the various species, a strongly disparate structure may be found, it is highly justified getting on with studying the pineal organ.

Materials and Methods

We have investigated the ultrastructure of the pineal body of doves *(Columba livia domestica)*, turkeys *(Meleagris gallopavo)* and ducks *(Anas domestica)* of different ages. For being investigated, our materials were fixed in osmium tetroxide buffered with collidine,

according to BENNETH and LUFT (1959), and then embedded in araldite after alcoholic dehydration. We used 3—4 p.c. uranyl acetate for contrasting the material, and lead citrate contrasting, according to REYNOLDS (1963) for staining the sections. The sections were made with ultramicrotome Porter—Blum LKB, and the microphotograms with electron microscope Tesla 242 D.

The electron microscopic investigations were aided by researchers IMRE ZS. NAGY (Institute for Biological Research, Tihany) and ÁRPÁD PÁRDUCZ (Electron Microscopic Laboratory of the Biophysical Institute, Biological Research Centre, Szeged).

Letter clue to the electron microscopic photographs

P	—	perikaryon	Mac	—	accumulation of mitochondria
Cm	—	cell membrane	Cr	—	mitochondrial crest
D	—	desmosome	Ly	—	lysosome (functioning)
Zo	—	zonula occludens	Lpr	—	prelysosome porthposome
Md	—	interdigitating membrane	Lpo		(residual body)
Ip	—	intercellular angle	Gr	—	granule
N	—	cell nucleus	V	—	vesicle
Nm	—	nuclear membrane	Pv	—	pinocytotic vesicle
Er	—	granulated endoplasmatic	Dv	—	dense core vesicle
Nm		reticulum	Bm	—	basal membrane
R	—	ribosome	E	—	endothelial cell
G	—	Golgi apparatus	Mc	—	macrophage
Gt	—	Golgi tubule	Ci	—	cilium
Gv	—	Golgi vesicle	Rci	—	radix cilii
Va	—	vacuole	Pr	—	protrusion
M	—	mitochondrium	Prv	—	protrusive vacuole
Mm	—	mitochondrium membrane	Mvb	—	multivesicular body
Ma	—	mitochondrium matrix	Myc	—	myelin configuration

Results

In the course of our investigations there occurred some interesting structural peculiarities that characterized the pineal organ of the birds investigated particularly and that have not been discussed in this direction so far. These are as follows:

1. Formation of the pineal cell membranes, 2. intracellular cavity systems, vacuole, 3. Golgi apparatus of vacuolar type with vesicles resp. granules of various types and sizes, 4. several lysosomes in transformation, 5. content of the apical surface and of the adjacent cavity system, 6. structure of the pineal vasculature.

Membrane formations

In the pineal organ of the three bird species investigated, between the cells of much the same diameter and building the follicles in more layers, membrane fusions resp. thickenings of more types and of very different length were to be seen. It is peculiar that often in the same cell two or three kinds of cell membrane formations were manifested. It is obvious, too, that the

course of the normal unit membrane is strongly wavy. The wideness of the intercellular space often changes (75 Å–160 Å). At the angles of cells, there are often wide polyhedric intercellular angles (Ip) to be found. At that time, the unit membrane divides, the intercellular space may grow eight to ten times wider forming polyhedric shapes of various forms and length. From among the membrane formations the following forms are characteristic:

Fig. 1. *Meleagris gallopavo:* formation of the membrane between the pineal cells (zonula occludens). x 35.000

Fig. 2. *Meleagris gallopavo:* formation of the membrane between the pineal cells (desmosome). x 30.000

1. A full fusion (Fig. 1) manifested generally in a long part. At that time, the unit membranes of cells fuse into a thin, dark line (Zo). This change in membrane has every criterion of the notion of occludent zone or closing crest. A fusion like that occurs the most frequently in the lateral walls of the long-shaped cells surrounding the lumen. It never appears near the blood vessels.

2. Desmosomal connection (Fig. 2). It is the most general and obvious thickening of the pineal cells (D). It manifests itself generally in a short region (about 0.6 u). At the desmosomes of pineal cells the intercelluiar space is wide and clear (150 Å). The electron dense filaments (tonofilaments) that can be seen well from the cytoplasm join with the membrane. The length of filaments reaches 400–500 Å. In a longer course there can often be obseved some desmosomal sectors where the intercellular space is dark. The adhesive filaments are usually still longer, 600–800 Å. The following two membrane formations are characterized by this form of thickening.

3. Interdigitating membrane system (Fig. 3). It is entirely peculiar that between the parenchymal cells there were found also some interdigitating cell borders that are characteristic of the cells of myocardium. It is distinctly visible in the picture that the unit membrane has formed large-sized vesicles with strangulations. The vacuoles of various sizes found in the cytoplasm of the cell get into a close connection with these. The interdigitating membrane system is connected with desmosomes in a longer sector.

4. Tubulovesicular connection (Fig. 4). We have often observed this peculiar formation in the deeper layer of follicles, close to blood vessels, in the first line of angular pineal cells of about identic diameter and in many layers. In that place the cell membranes fused long and strongly divide at the angles of cells, forming the particular tubulovesicular system (Tv) seen in the picture. The tubular system may be produced by the digital protrusions of the contiguous cell membranes reaching one another. At the beginning we thought that the canals of the rough endoplasmatic reticulum of the adjacent cells were connected together in these places but that was not possible because the ribosome never appeared on the surface of canal. It becomes clear after investigating the connections that a formation of membrane is in question. This structure that is similar to the biliary capillaries is referring to an absorbing resp. evacuating function. A particular attention is also deserved by the long fused double membrane where the intercellular space completely disappears but from the cytoplasm a filament similar to that of the desmosomes is adhering to the membrane. This dark desmosomal connection extends almost over the entire limiting membrane, interrupted only at the tubulovesicular systems.

The close connection between the pineal cells can be found not only at the pineal cells of birds but also at those of mammals. They were noticed first by HOPSU and ARSTILA (1965) between the pinealocytes of the rat and published under the name somato-somatical synapsis. The authors mentioned above have not found any divergent forms. As according to the literary data there appear occludent zonules between the photoreceptor cells of the retina (DOWLING and BOYKOTT, 1967; DOWLING and GIBBONS, 1962; EAKIN, 1965; HOLMBERG, 1969; 1971) and desmosomal connection between the ependymal cells (BLOOM FAWCETT, 1970), the membrane mutations observed do not give us any argument for deciding the proper place of the avian pineal cells. At any rate, it is sure that the matter in question is a parenchymal tissue of a very close functional collaboration. This is a particularly close functional cennection in the vicinity of blood vessels. The complete fusions, as known well, are meaning

an almost perfect barrier for the macromolecular matters. It is probable that these close fusions extending over a large surface do play an important part among the lumen content and the materials of the blood vessels lying deeper.

Vacuolar cavity systems (Va)

In some of the avian pineal systems there appeared some major cavity systems (Fig. 5). Sometimes the cavity filled up the cell even to the half. These may be vesicles filled in with some fluid. As shown by the figure,

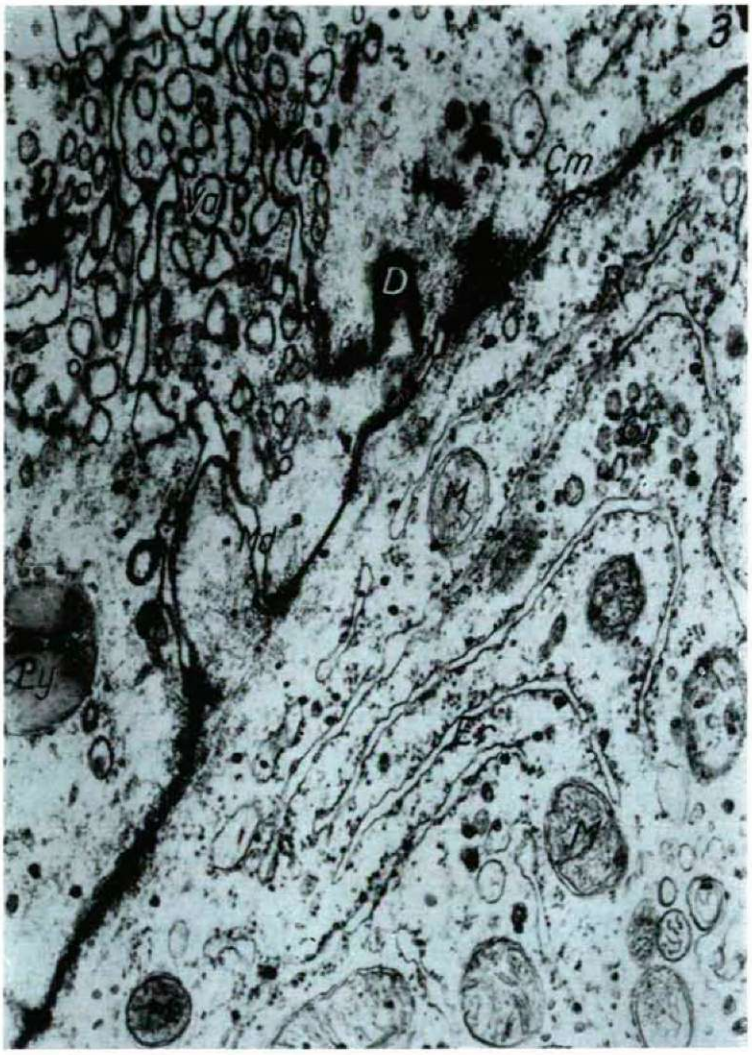

Fig. 3. *Columba domestica:* interdigitating membrane with detaching vesicles. x 35.000

the vacuole is lying in the immediate neighbourhood of the nucleus. These vacuoles of major size may be in connection with lesser vacuolar groupings multiplied in the cytoplasm everywhere but particularly round some major vacuoles and directed towards one another with their protrusions. To be sure, it is possible, as well, that these lesser cavities are the vesicles broken off the large cistern.

In the world of living, the vacuoles of fluid are mainly known in the vegetable cells. From the cells of the animal kingdom they are almost entirely

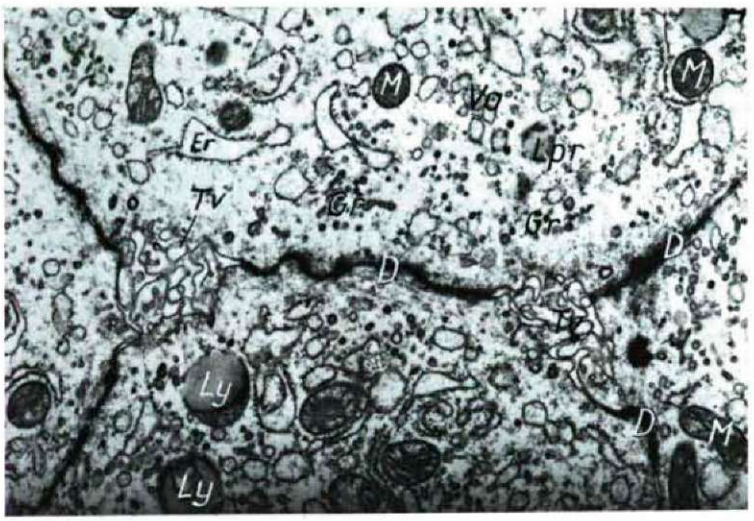

Fig. 4. *Columba domestica:* tubulo-vesicular connection between the pineal cells. x 17.600

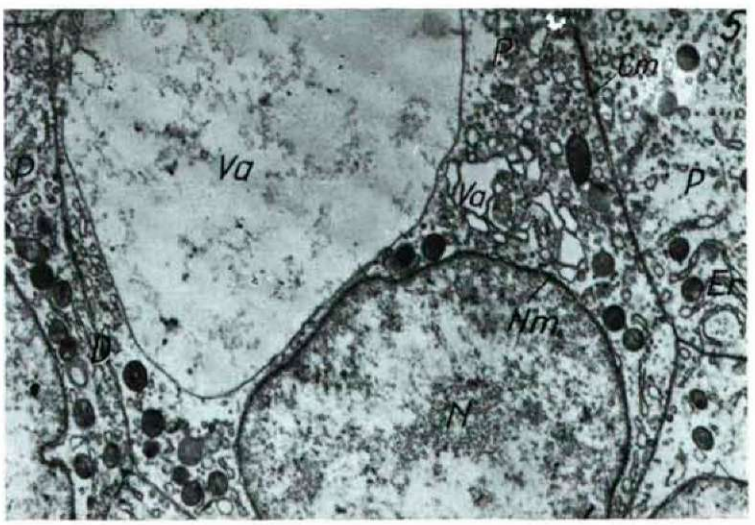

Fig. 5. *Columba domestica:* a large-sized vacuole in the pineal cell. x 17.600

absent. We find only according to the recent literary data some reference to, that there occurred some cavity systems similar to the above mentioned ones in the venticular area of brain, as well, in the ependymal cells containing water (VIGH–TEICHMANN and VIGH, 1969).

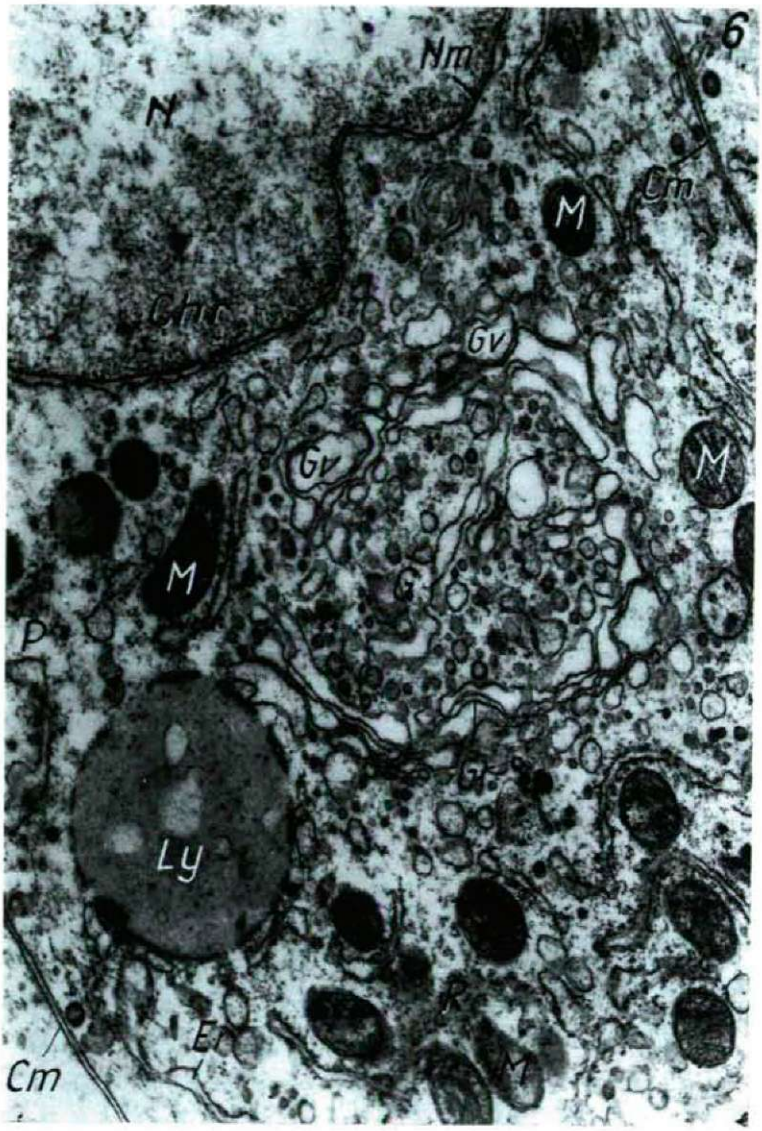

Fig. 6. *Columba domestica*: GOLGI apparatus of the pineal cell. x 22.000

Golgi apparatus (G)

The GOLGI apparatus is mostly present in the form of oblong, narrow vessels (Gv) and of small vesicles detached at their ends (Gv) in the cells. This form was never found in the perikaryon of the parenchymal cells of avain pineal body. Instead of these, a peculiar vacuolar type can be seen (Fig. 6). The Golgi apparatuses are very well developed in the pineal parenchymal cells. Apart from some flat canals at their edges – that perceptibly enter, anyway, the centre, too, – this apparatus is built by very large-sized vesicles of diameters 2500–3500 Å. In the by and large circular apparatus, rich in vacuoles of very various sizes, some vesicles and granules of different size and colour are to be seen. The dark granules are frequent. Among these there are many completely dark granules of about 250–300 Å. In addition to these, there appear also some larger and a little clearer granules of a size 450 Å in a large enough number. From among the dark granules, particularly those of smaller size appeared very frequently even in the cytoplasm scattered, mainly near the endoplasmic reticulum and along the cell membranes, as well. Rather rarely, there appeared vesicles of dense core type, too, sized in the average 800 Å. Besides the dark granules, also a great number of clear vesicles can be found in the Golgi apparatus. The diameter of these is 400–600 Å. They are, as a rule, round but there occur elliptical forms, too. From among the elliptical forms, those of larger size have diameters 850 Å in length and 400 Å in width. In the cytoplasm the clear vesicles are rare. In the vicinity of cell membranes (Cm) they appear usually as pinocytotic vesicle types and they are particularly frequent in the endothelial cells of the blood vessels. There occurred, even if scarcely, also some multivesicular bodies in the GOLGI apparatus, with diameters of 2500 Å. Similar forms were sometimes found in the cytoplasm, too, most of them having appeared in the content of the follicular lumen.

We had first thought the Golgi apparatus of vacuolar type to be some vesicle containing or storing fluid. It was decided only by its vicinity to the cell nucleus, its granulosity and permanent uniform appearance that the matter in question is the GOLGI apparatus. We have often observed lysosomes in the neighbourhood of the GOLGI apparatus and also the open, resp. vacuolarly dilated canals of the endoplasmic reticulum manifested themselves immediately beside the Golgi apparatus.

Lysosomes (Lv)

The lysosomes, these electron microscopic cell-organelles of heterogeneous appearance and function are probably permanent and large-size cell-organelles in the pineal cells of the species investigated. According to their appearance, they are vesicles surrounded by a unit membrane. They are interesting because of their alternating electron density and different size. We regard the lysosomes of the parenchymal cells of the bird as lysosomes rich in hydrolytic enzymes. We could follow even the most different transformed forms of lysosomes. In the cells investigated we have found a great number of forms, from the quite small organelles of 150 mμ till the lysosome of about 3 μ. We consider as prelysosome (Lpr) the small – mostly homogeneous – form (Fig. 7). There

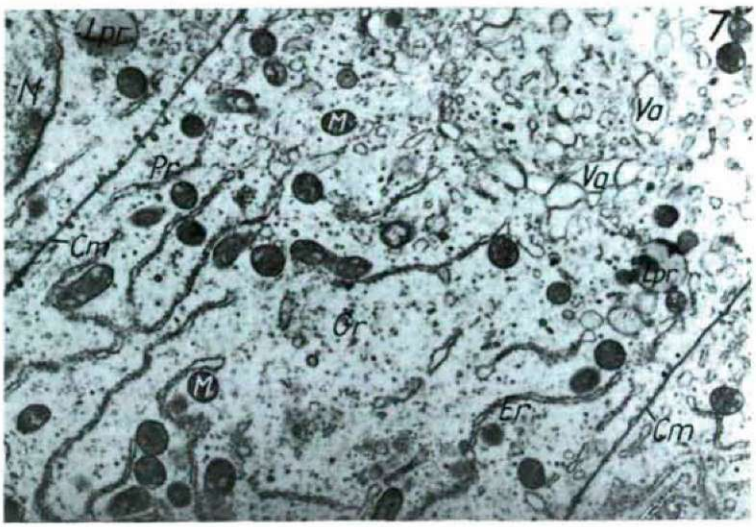

Fig. 7. *Anas domestica:* lysosome in the pineal cell (prelysosome). x 17.600

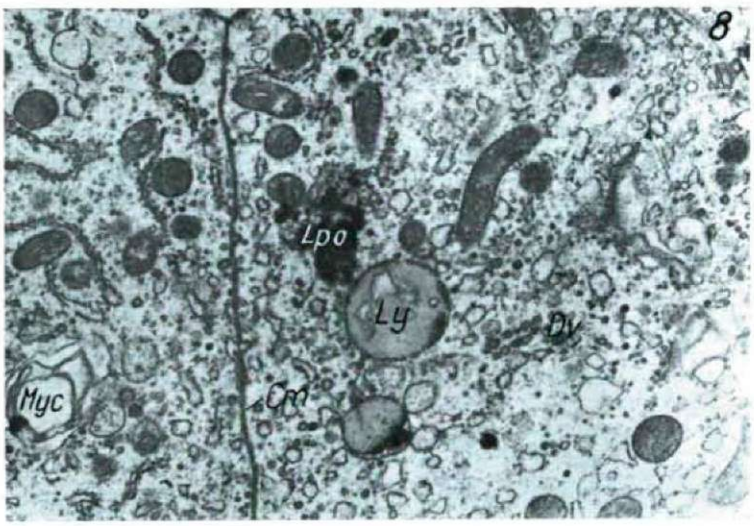

Fig. 8. *Meleagris gallopavo:* residual body in the pineal cell. x 17.600

develop from these the real or proper lysosomes of spotted structure, cap-like loaded with electron dense nodules at the edge or peak (Figs. 6, 8). The latter ones have appeared in the largest number in the pineal cells. They are striking in almost every picture (Ly). In their vicinity we could always observe a dilated, granulated endoplasmic reticulum and a great many pinocytotic vesicles. However in lower number, the residual bodies are nevertheless very conspicuous; as post-lysosomal forms (Lpo), they are probably storing the decomposition products of pineal cells (Fig. 8). In the body surrounded with a membrane, decomposing

dark granules are to be seen. The electron dense granules reminding of melanin or lipofuchsin granules are actually stuffing the matrix of lysosome. There has never appeared any autophagosome containing any cellular element (mitochondrium or granule). We have not observed, in a single case, either, that these residual forms would ever have been discharged by exocytosis throught the cell membrane.

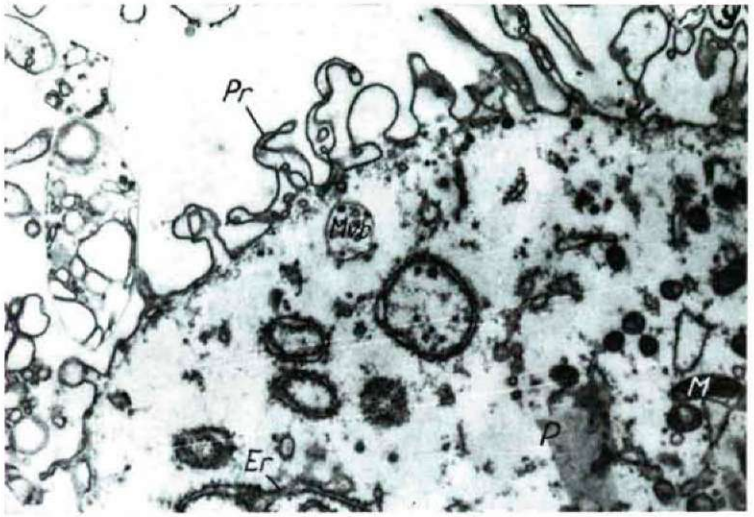

Fig. 9. *Columba domestica* (8-day old): luminal surface of the cell limiting the follicle. Protrusions. x 35.000

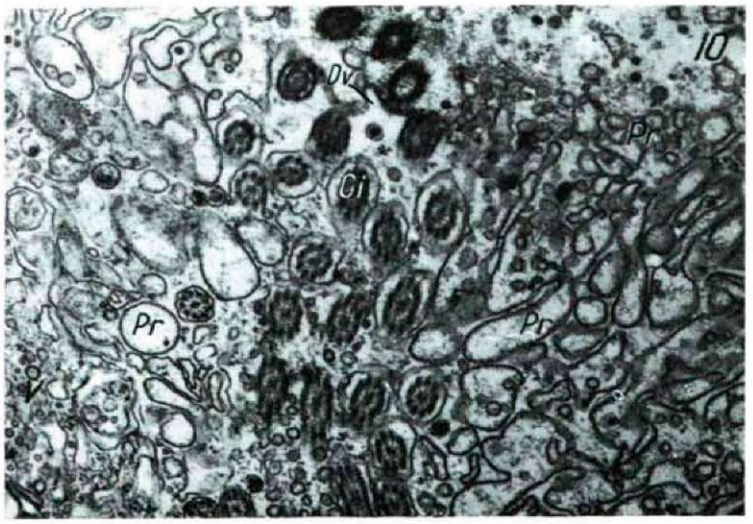

Fig. 10. *Columba domestica* (6-month old): surface of the follicle-limiting cell in cross-section. Cross-sections of protrusions and cilia. x 35.000

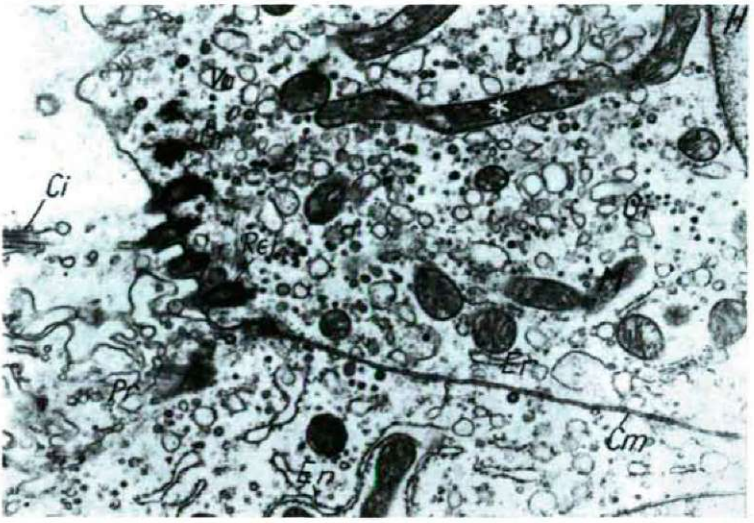

Fig. 11. *Meleagris gallopavo:* surface of the follicle-limiting cells. Radices of cilia. x 35.000 x 35.000

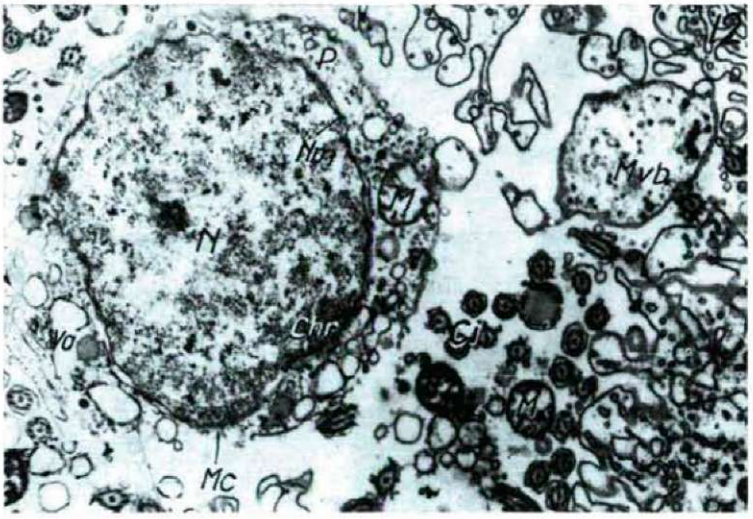

Fig. 12. *Columba domestica:* content of the pineal follicular lumen. Macrophage. x 17.600

Surface and lumen content of the pineal cells

The pineal bodies of all the three bird species investigated are of follicular structure. Protrusions and cilia are reaching down into the lumen of the follicle (Figs. 9 to 12). The protrusions are peculiar twisted formations, filled in with tiny clear vesicles. The matter in question is probably containing pinocytotic vesicles and the essential structural elements of the secernent function of the

pinael cells. We have observed that at the end of protrusions even larger vesicles and protruding pieces, as well, may be detached. The lumen is full of these detached pieces as well as of mitochondria devoted to destruction, of small-sized lysosomes and quite well multivesicular bodies. There often appeared laminated systems similar to myelinic configurations and some independent cells – probably some macrophages – as well (Fig. 12).

Fig. 13. *Columba domestica:* pineal capillary cross-section. Peculiarly developed basal membrane systems and perivascular spaces. x 17.600

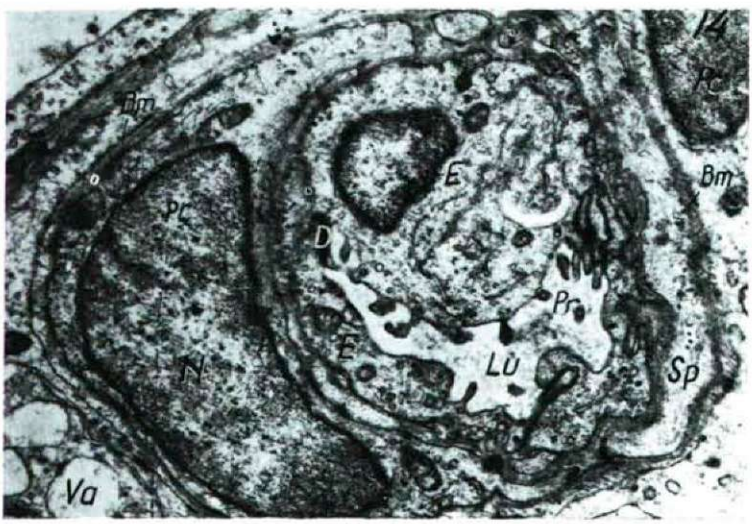

Fig. 14. *Anas domestica:* pineal capillary cross-section. Pinocytotic vesicles and protrusions. An endothelial cell protruding into the lumen. x 22.000

According to our observation, on the surface of the pineal cells, in quite young birds (eight-day old doves) there are only some protrusions (Fig. 9), the cilia appeared only later, after the sixth week (Fig. 10). They usually appear mixed on a cell surface. There are, however, some cell surface only with protrusions and others only with cilia. On this basis, in our opinion, there can ot be told any cells from another. We regard the cilia to have a typical 9 + 2 structure, adhering with conspicuously strong ciliary roots (Rci) to the surface of cell (Fig. 11). Generally a great many vesicles and granules and particularly elongated mitochondria take place in the cytoplasm close to the cell surface.

Blood supply of the pineal cells

The blood vessels between the pineal cells are showing electron microscopically a peculiar structural arrangement, most part of them being small arteries resp. capillaries and they are present, as a rule, only between angular parenchymal cells of equal diameters and of deeper site. The monostratal, long-shaped cells limiting the lumen haven't any independent capillary system more. A characteristic of the pineal small vessels is the obvious thick and dark basal membrane system (Figs. 16, 17). Round the cross-section of one or two blood vessels there are often to be seen double or triple basal membranes with considerable perivascular spaces. The nerve fibres that are of sympathetic origin have only appeared here.

The wall of capillaries is mostly constructed of quite thin endothelial cells attached to each other with desmosomes. The desmosomes between the endothelial cells are long, with thick electron dense filamens and a clear but narrow intercellular space. The cytoplasm of the endothelial cells does contain the general cellular elements and we have not found any difference as compared with the endothelial cells of other capillaries. The nucleus is markedly large and strongly granulated, being in most cases also indented. There are, to be sure, polygonal endothelial cells of larger size, too, reaching into the vascular lumen. It may be supposed about these cells that they play the part of valve in the regulation of blood flow. It is possible that these are characteristic only of the venous capillaries. Apart from the basal membrane system, also the rich protrusive system is a peculiarity of the pineal endothelial cells. From the surface of endothelial cells a great many protrusions reach into the lumen, respectively some empty vacuoles of different sizes may be seen as detached from the protrusions and forming groups either in the lumen or in the perivascular space. Seeing these formations, we think with reason on a particular secretory function of merocrine character.

The large number of pericytes beside the capillaries is remarkable, as well. There may be distinguished two types of these pericytes. At one of the types, the strongly indented cellular nucleus is dark, granulated, the nuclear membrane is smooth and there are to be seen never any nuclear pores. The perikaryon is very narrow, creating many protrusions. At the other type of pericytes a long-shaped nucleus can be seen, with a permanently smooth surface. At these, the cytoplasmic cell part is much larger and wider and they have fewer cellular protrusions. It may be supposed that the two types of cells play two kinds of functions. It is probable that the first one takes a prominent part in contraction and the second one in support.

References

AXELROD, J., WURTMAN, R. J. (1966): Die Zirbeldrüse als biologische Uhr. — Umschau *66*, 158—159.

AXELROD, J., WURTMAN, R. J. and WINGET, CH. M. (1964): Melatonin synthesis in the hen pineal gland and its control by light. — Nature (Lond.) *201*, 1134.

BARGMANN, W. (1943): Die Epiphysis cerebri. In: Hb. der mikr. Anatomie des Menschen, — Bd. VI/4. Berlin.

BENNETH, G. H. and LUFT, J. H. (1959): Collidine basis for buttering fixatives. — J. Biophys. Biochem. Cytol. *6*, 113—114.

BISCHOFF, M. B. (1967): Ultrastructural evidence for secretory and photoreceptor functions in the avian pineal organ. — J. Cell. Biol. *35*, 13—14.

BISCHOFF, M. B. (1969): Photoreceptoral and secretory structures in the avian pineal organ. — J. Ultrastruct. Res. *28*, 16—26.

COLLIN, J. P. (1966a): Contribution a étude de l'épiphyse embryonnaire d'oiseau. — C. R, Acad. Sci. (Paris) *262*, sér. D, 2263—2266.

COLLIN, J. P. (1966b) Étude préliminaire des photorécepteurs rudimentaires de l'épiphyse de *Pica pica* L. pendant la pie embryonnaire et postembryonnaire. — C. R. Acad. Sci. (Paris) *263*, 660—663.

COLLIN, J. P.: (1966c) Sur l'évolution des photorécepteurs rudimentaires épiphysaires chez la pie (*Pica pica* L.). — C. R. Soc. Biol. (Paris) *160*, 1876—1880.

COLLIN, J. P. (1967a): Le photorécepteur rudimentaire de l'épiphyse d'oiseau: le prolongement basal chez le passereau *Pica pica* L. — C. R. Acad. Sci. (Paris) *265*, 48—51.

COLLIN, J. P. (1967b): Nouvelles remarques sur l'épiphyse de quelques lacertiliens et oiseaux. — C. R. Acad. Sci. (Paris) *265*, 1725—1728.

DODT, E., UECK, M., OKSCHE, A. (1969): Relation of structure and function. The pineal organ of lower vertebrates. — J. E. Purkyne Centenary Symposium, Prag.

DOWLING J. E. and BOYCOTT, B. B. (1967): Organization of the primate retina: electron microscopy. — Proc. roy. Soc. B. *166*, 80—111.

DOWLING J. E. and GIBBONS, I. R. (1962): The fine structure of the pigment epithelium in the albino rat. — J. Cell. Biol. *14*, 459—474.

FUJIE, E. (1968): Ultrastructure of the pineal body of the domestic chicken, with special reference to the changes induced by altered photoperiods. Arch. histol. jap. *29*, 271—303.

EAKIN, R. M. (1965): Differentiation of rods and cones in total darkness. J. Cell Biol. *25*, 162—165.

GASTON, S., MENAKER, M. (1968): Pineal function: The biological clock in the sparrow? Science *160*, 1125—1127.

KAPPERS, ARIENS J. (1971): The pineal organ: An introduction. The pineal gland. A Ciba Foundation Symposium. p. 3—34. Edinburgh and London: Churchill-Livingstone.

KELLY, D. E. (1962): Pineal organs: photoreception, secretion, and development. Amer. Sci. *50*, 597—625.

KOBAYASHI, H. (1969): Pineal and gonadal activity in birds. In: Seminar on hypothalamic and endocrine functions in birds (Tokyo, May 19—24, 1969). Abstract (eds. H. KOBAYASHI and D. S. FARNER), p. 72. Tokyo.

HOLMBERG, K. (1969): Hagfish eye: ultrastructure of retinal cells. Acta zool. (Stockh.) *50*, 179—183.

HOLMBERG, K. (1971): The hagfish retina: Electron microscopic study comparing receptor and epithelial cells in the pacific hagfish, Polistotrema stouti, with those in the atlantic hagfisch, Myxine glutinosa. — Z. Zellforsch. *121*, 249—269.

MORITA, Y. (1966): Absence of electrical activity of the pigeon's pineal organ in response to light. — Experientia (Basel) *22*, 402.

OKSCHE, A. (1968): Zur Frage extraretinaler Photorezeptoren im Pinealorgan der Vögel. — Arch. Anat. (Strasbourg) *51*, 497—507.

OKSCHE, A., KIRSCHSTEIN, H. (1969): Elektronenmikroskopische Untersuchungen am Pinealorgan von *Passer domesticus*. — Z. Zellforsch. *102*, 214—241.

OKSCHE, A., KIRSCHSTEIN, H., KOBAYASHI, H., FARNER, D. S. (1972): Electron microscopic and experimental studies of the pineal organ in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*. — Z. Zellforsch. *124*, 247—274.

OKSCHE, A., MORITA, Y., VAUPEL von HARNACK, M. (1969): Zur Feinstructur und Funktion des Pinealorgans der Taube (*Columba livia*) — Z. Zellforsch. *102*, 1—30.

OKSCHE, A., VAUPEL—von HARNACK, M. (1966): Elektronenmikroskopische Untersuchungen zur Frage der Sinneszellen im Pinealorgan der Vögel. — Z. Zellforsch. *69*, 41—60.

QUAY, W. B. (1965): Histological structure and cytology of the pineal organ in birds and mammals. In: J. ARIENS KAPPERS and J. P. SCHADÉ, Progress in brain research, *10*, 49—86. Amsterdam—London—New York: Elsevier Publ. Co.

QUAY, W. B. (1966): Rhythmic and light-induced changes in levels of pineal 5-hydroxindoles in the pigeon (*Columba livia*). Gen. comp. Endocr. *6*, 371—377.

QUAY, W. B., RENZONI, A. (1963): Comparative and experimental studies of pineals structure and cytology in passeriform birds. Riv. Biol. 56, 393—407.

RALPH, C. L., DAWSON, D. C. (1968): Failure of the pineal body of two species of birds (*Coturnix coturnix japonica* and *Passer domesticus*) to show electrical responeses to illumination. — Experientia (Basel) *24*, 147—148.

RENZONI, A., EAKIN, R. M., QUAY, W. B. (1968): Cilia of modified structure in avian pineal organs, — Fourth European Regional Conference on Electron Microscopy, Rome. 563—564.

REYNOLDS, E. S. (1963): The use of lead citrate at high pH as an electronopaque stain in electron microscopy. — J. Cell. Biol. *17*, 208—212.

UECK, M. (1970): Weitere Untersuchungen zur Feinstruktur und Innervation des Pinealorgans von *Passer domesticus*. — Z. Zellforsch. *105*, 276—302.

VIGH, B., and VIGH—TEICHMANN, I. and Aros B. (1971): Ultrastructur der Liquorkontaktneurone des Zentralkanals des Rückenmarkes von Karpfen (*Cyprinus carpio*) — Z. Zellforsch. *122*, 301—310.

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EFFECTS OF LANTHANUM AND ALKALINE EARTH METAL IONS ON THE POTASSIUM CONTRACTURE OF THE HELIX HEART

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It is known that the heart muscle of molluscs differs in several respects from that of vertebrates. It will suffice to refer from among these to the structure of the heart muscle, the differences demonstrated in its biochemical composition, the presence of a diffuse pacemaker, the different responses to electric stimulation, the difference in excitatory mediators, as well as to the different characters of some ion effects. At the same time, in other relations, some similar features can be recognized, too. Worthy of note, from this point of view, are the myogenic automatism, the inhibiting effect of ACH, the presence of a Na–Ca-dependent action potential, the depolarizing effect of potassium and the contracture connected with this.

On the basis of all this, it is justified to raise the question of the regularities of structure and functioning of the excitation-contraction coupling. From this point of view, data cennected with the problem of the potassium contracture and its ion-dependence, have been published by OZEKI (1964), and HILI, GREENBERG, IRISAWA, NOMURA (1970) with regard to the pharynx retractor or radula protractor muscle of two snail species, and by NOMURA (1965) and BURTON and LOUDON (1972) with regard to the heart of *Dolabella* and *Helix*. It can be established from these investigations that the K-depolarization of molluscan muscles and the associated contracture, and also the Ca-dependence of the phenomenon are similar to those reported in vertebrate organs and particularly in the heart of the frog (NIEDERGERKE, 1956; 1963; LAMB and MCGUIGAIN, 1966).

The ion-dependence $(Ca) : (Na)^2$, known from the works of WILBRANDT and KOLLER (1948), LÜTTGAU and NIEDERGERKE (1958) on the contractility of frog heart, is not valid for the molluscan heart (NOMURA, 1965; BURTON and LOUDON, 1972). According to the investigations of the authors cited, the effect of magnesium on the molluscan heart is similar to that of sodium on the frog heart.

In the calcium-dependent potassium contracture the problem of the replacement of calcium by various cations seems to be rather contradictory in the literature. It is sufficient in this connection to refer to two data. FRANK (1962) found many bi- and trivalent cations to be calcium-substitutive in the potassium contracture in the skeletal muscle of the frog. At the same time, GAINER (1968)

could not substitute essentially similar cations for the specific role of calcium in the muscle of the lobster.

It is known, mainly from investigations on vertebrate muscles, that lanthanum can interact with the Ca^{++}-binding sites or stores on the membrane surface, in this way being able to inhibit the calcium-dependent potassium contracture (SANBORN and LANGER, 1970; GOODMAN and WEISS, 1971; and others).

In this paper a study is made, in connection with the potassium contracture, of the problem of calcium substitution, and of the effect of lanthanum on the excitation-contraction coupling of the edible snail heart.

Materials and Methods

Physiological experiments: Recordings were made of the isotonic contractions of the isolated *Heltix pomatia* hearts, hung up in an organ vessel and extended with a 1,3 g weight, on a smoked kymograph drum. Solutions of the following composition were used:

Helix—Ringer (according to Jullien—Ripplinger) NaCl 111,1 mM, KCl 1,87 mM, $CaCl_2$ 1,08 mM, $NaHCO_3$ 2,39 mM pH = 7,4

K—Ringer

KCl	10	15	20	30	50	100	mM
NaCl	101,1	96,1	91,1	81,1	61,1	11,1	mM
$CaCl_2$	1,08	1,08	1,08	1,08	1,08	1,08	mM
$NaHCO_3$	2,39	2,39	2,39	2,39	2,39	2,39	mM

For the investigation of the calcium-dependence of the potassium contracture, as well as the possibility of calcium-substitution, the contracture elicited by 50 mM K-Ringer was considered. Not more than 5—10% difference was obtained in the height of the contractions, even after eliciting contractures with solution three times successively in the same heart (taking into consideration a ten-minute washing interval).

In the course of the experiments, the snail hearts were equilibrated in Helix—Ringer under oxigenated conditions for 20 minutes, and then control contraction was recorded in 50 mM K-Ringer. After washing, the hearts were pretreated for 5 to 30 minutes in a solution (changed several times) containing the appropriate concentration (0—1 mM) of calcium or calcium-substitute, and a contraction was again elicited with a solution containing calcium the same concentration as the incubating solution or calcium-substitute and 50 mM KCl. The relation of the two contractions was evaluated. In one heart, always only one control and one experimental reaction were recorded. Generally the average of at least three parallel experiments was taken into consideration. In the course of the experiments solutions of the following composition were used:

Ca-Ringer

$CaCl_2$	1	0,8	0,5	0,4	0,3	0,2	0,1	0	mM
NaCl	111,1	111,1	111,1	111,1	111,1	111,1	111,1	111,1	mM
KCl	1,87	1,87	1,87	1,87	1,87	1,87	1,87	1,87	mM
$NaHCO_3$	2,39	2,39	2,39	2,39	2,39	2,39	2,39	2,39	mM
EDTA	—	—	—	—	—	—	—	1,5	mM

The composition of the Ca-50 mM K-Ringer always conforms to the calcium content of the corresponding Ringer solution, but the concentration of NaCl was only 61,1 mM.

The compositions of the 1, 0,8, 0,5, 0,4, 0,3, 0,2, 0,1 mM barium, strontium, magnesium Ringers, as well as that of the alkaline earth metal ion-50 mM K-Ringer of the corresponding concentration were formed in a completely analogous way as described for calcium. In the case of strontium $NaHCO_3$ was not used.

In the experiments carried out with $LaCl_3$, the compositions of the 1 mM Ca-Ringer and 1 mM Ca-50 mM K-Ringer were taken into consideration, with the difference that $LaCl_3$ was also added to the solutions, in a quantity of 0,005, 0,02, 0,2, 0,5 or 1 mM and $NaHCO_3$ was not used. The pretreatment in the La-1 mM Ca-Ringer lasted for five minutes.

Isotope experiments: A study was made, palallel with the physiological experiments, of the ^{140}Ba uptake of *Helix* hearts and the washing out of the isotope. The isotope experiments were performed in the same way as the physiological investigations, in 30 ml 0,3 or 0,1 mM inactive Ba-Ringer, mixed with about 0,6 μCi/ml ^{140}Ba (pH = 7.4, 22 °C). The isotope uptake of the hearts and the depolarizing effect of 50 mM KCl on the development of the uptake were then examined. The uptake is given as a percentage of the incubating solution, referred to 1 mg dry weight.

After a 45-min. uptake the hearts were washed in 30 ml Ca-free Ringer, for 3 × 1 min. The washing out of the isotope in 30 ml Ca-free Ringer was evaluated on the basis of the change in activity (count/minute/ml). The effect of Ca^{++} on the ^{140}Ba washing out was investigated. ^{140}Ba was detected via its gamma-radiation, in 0,1 or 0,2 ml organ-bath samples.

Electron-microscopic investigations: The electron-microscopic procedure was made in the same way as described in an earlier publication, after being fixed in glutaraldehyde-osmium as usual (ERDÉLYI—HALÁSZ, 1972).

Results

In the first series of experiments we worked with animals of 3 cm shell diameter, investigating the effect of K-Ringers of various concentrations on the contracture of the heart muscle. The results obtained are shown in Fig. 1, taking into consideration the average of five different hearts. In the graph the height of contracture is shown measured in mm, after a contracture period of 1 minute, as a function of the extracellular potassium concentration indicated on the logarithmic scale. The results, in spite of the different Helix–Ringer compositions and the other system of recording, can be compared well with the data achieved by BURTON and LOUDON (1972) for membrane depolarization and potassium contracture.

The contracture observed in the 50 mK K-Ringer was also found by us to be rather of a phasic character, in contrast with the barium contracture described in our earlier publications, that proved to be of a markedly tonic character (ERDÉLYI, 1971). There is also a considerable difference in that the spontaneous contraction generally undergoes a pause during the potassium contracture, whereas in the case of barium contracture it may change but in continues nonetheless. The contracture elicited by 50 mK K-Ringer attains its peak value in about 30–50 sec and, after a short plateau, begins a spontaneous ralaxation after the end of the second minute. This fast decreasing section of the initial phase turns at 50% relaxation into a relaxation of slowly decreasing tone. As compared to the fast period, the slow one lasts 5–10 times longer, reaching the base level only very slowly. Such a high insentivity then develops that, after a ten-minute washing, no contraction can be elicited in a new 50 mM K-Ringer, or only a contraction of very low degree. The spontaneous decrease in the potassium contracture was analysed by NIEDERGERKE (1965) in the heart of the frog. It is probable that similar factors may play a part in inducing the phenomenon here too.

After a fluid exchange within the two-minute contraction period, and after the insertion of a ten-minute washing pause the potassium insensitivity is not manifested. Under these conditions, there is no more than a 5–10% decrease in the height of the contraction elicited subsequently three-four times.

In the next experimental series we investigated the calcium-dependence of the potassium contracture and the problem of the possibility of substituting alkaline earth metal ions for calcium in the process. In this experimental series

we worked with animals of 4.5 cm shell diameter. The results obtained are shown in Fig. 2. The decrease in the extracellular calcium concentration is followed by a decrease in the potassium contracture to 1–0 mM and by its complete cessation, corresponding to the Ca-dependence of the phenomenon. At the various potassium concentrations in the heart of the frog and snail, as well as in the radula protractor muscle of *Busycon,* a contraction curve of

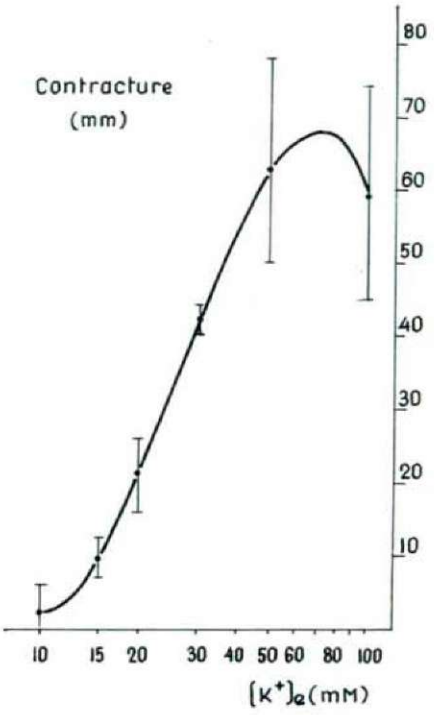

Fig. 1

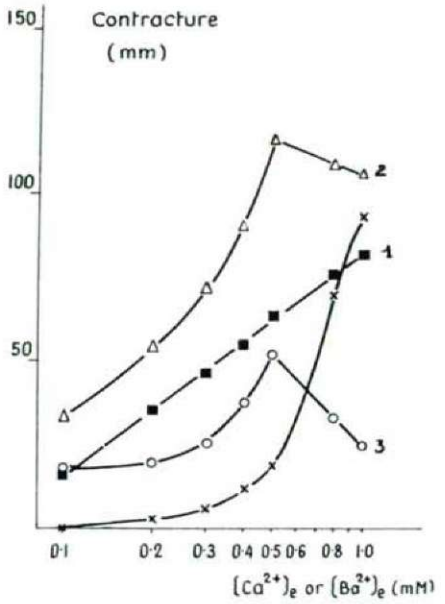

Fig. 2

Fig. 1. Effect of the extracellular potassium concentration on the contracture of the *Helix* heart muscle. Every point is a mean value of contractions measured on five different hearts. The vertical lines indicate the height of the minimum and maximum contractures obtained in five measurements.

Fig. 2. Change in the contracture elicitable by 50 mM K-Ringer, plotted against the concentration $(Ca^{2+})e$, and $(Ba^{2+})e$. X = change in the contracture as a function of the extracellular calcium concentration. o = change in the contracture as a function of the extracellular barium concentration, i.e. the difference of the two latter curves. □ = level of tonic contracture in Ba-Ringer of different concentrations, at the end of the 30-minute pre-incubation. △ = level of contracture, in the corresponding Ba-50 mM K-Ringer. Every measurement is the mean value of the contractions obtained in at least three different hearts. The shell diameter of the animals used in the experimental series was 4.5 cm. The size of the contraction in the 1 mM Ca-50 mM K-Ringer, considered as control, was obtained from the average of 10 measurements as 92 mm, S.D. ± 13.

Fig. 3. Myofiber of the *Helix* heart ventricle in longitudinal (A, B) and transverse (C) sections. The electron micrograph shows the structure of the sarcotubular system. M = mitochondrium, SR = transverse and longitudinal tubules of the sarcoplasmic reticulum, S = sarcolemmal infoldings, T = dilated subsarcolemmal vacuoles, touching the membrane surface, Z = Z-material, mf = myofilaments. Glutaraldehyde-osmium.

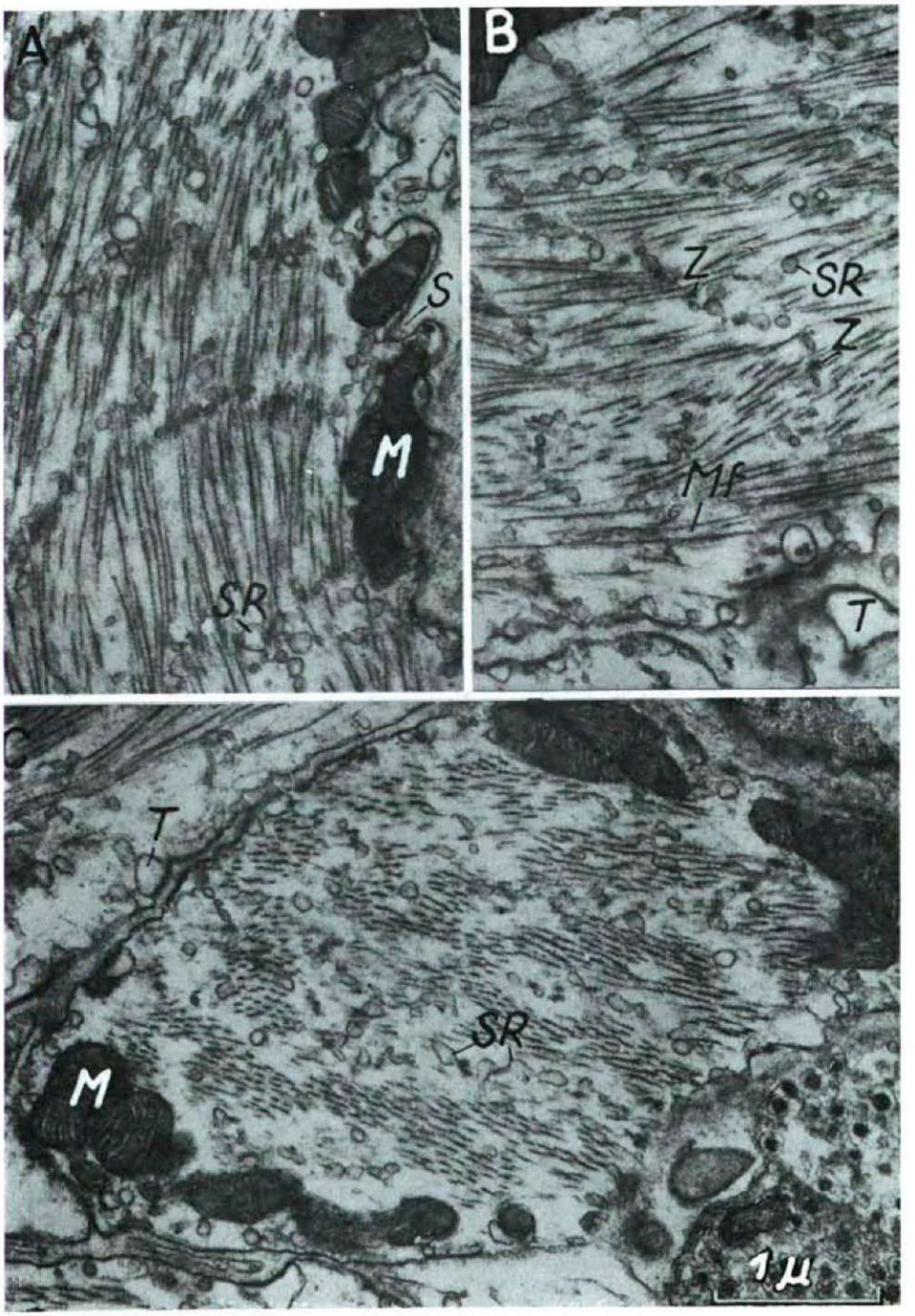

Fig. 3

similar type may be recorded as a function of the extracellular calcium concentration (LAMB and MCGUIGAIN, 1966; HILL, GREENBERG, IRISAWA, NOMURA, 1970; BURTON and LOUDON, 1972). All this shows the possibility of a similar mechanism from the point of view of the phenomenon. According to the generally accepted opinion, the rise of the ionic calcium level within the muscle fibre is the contraction-inducing key factor. On the other hand, the release of the sarcoplasmic calcium is influenced by the state of the Ca^{++}-binding sites on the outer surface of the membrane (GAINER, 1968 and others). The morphological basis for the function of both systems in the vertebrate skeletal and

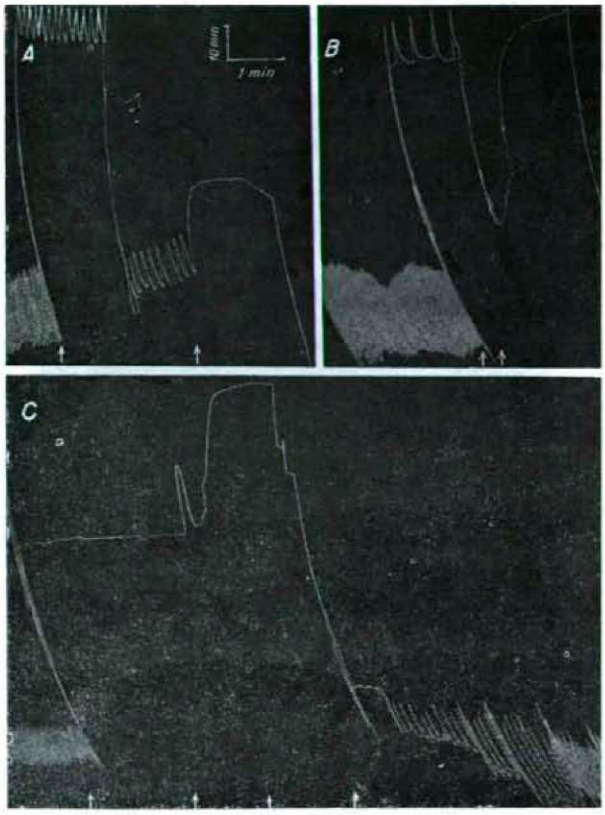

Fig. 4. Change in the contracture elicitable by 50 mM KCl, plotted against the extracellular barium concentration.
A. First arrow: pretreatment in 1 mM Ba-Ringer for 30 minutes. At the end of the thirtieth minute, during recording of the spontaneous contraction, the level of the pen arm was artifically lowered. Second arrow: 1 mM Ba-50 mM K-Ringer.
B. First arrow: pretreatment in 0.5 mM Ba-Ringer for 30 minutes. At the end of the spontaneous contractions recorded from the thirtieth minute the level of the pen arm was artifically lowered. Second arrow: 0.5 mM Ba-50 mM K-Ringer.
C. First arrow: pretreatment in 0.3 mM Ba-Ringer for 30 minutes. Second arrow: 0.3 mM Ba-50 K-Ringer. Third arrow: beginning of washing in Ca-free Ringer. Fourth arrow: washing in 4 mM Ca-Ringer. Parallel with the physiological experiments the ^{140}Ba uptake of the heart and the washing out of the isotope taken up were investigated, on the basis of the activity change in the 0.1, and 0.2 ml samples of the bath.

heart muscles is provided by the T-system, ensuring the connection with the sarcolemma, and by the sarcoplasmic reticulum (Phyiol. Symp. 1965, PHILPOTT and GOLDSTEIN, 1967, and others). It is known from NORTH's (1963) publication that the myocardium of *Helix aspersa* has a very rich sarcotubular system. Practically the same is shown by the electron-micrographs in Fig. 3, as well as by SCHLOTE's (1964) Figures with regard to the heart of *Helix pomatia,* too. In the longitudinal and transverse sections reported, the transversal and longitudinal arrangement ot the very richly developed sarcotubular system can be well seen. The connection of the sarcotubular system with the sarcolemma is very conspicuous here too. Sarcolemmal infoldings associated with more dilated subsarcolemmal vesicles can be observed sporadically. In some places, a connection between the characteristic Z-granules and the sarcotubular system can similarly be observed. On the basis of all this, it may be stated that in the heart of the *Helix,* in harmony with the physiological results, the organization of the muscle membrane and the sarcotubular system may be compared, in a less differentiated form, to those described in the vertebrate skaletal and heart muscles. There is, therefore, in essence a suitable morphological basis for comparing the similar physiological phenomena.

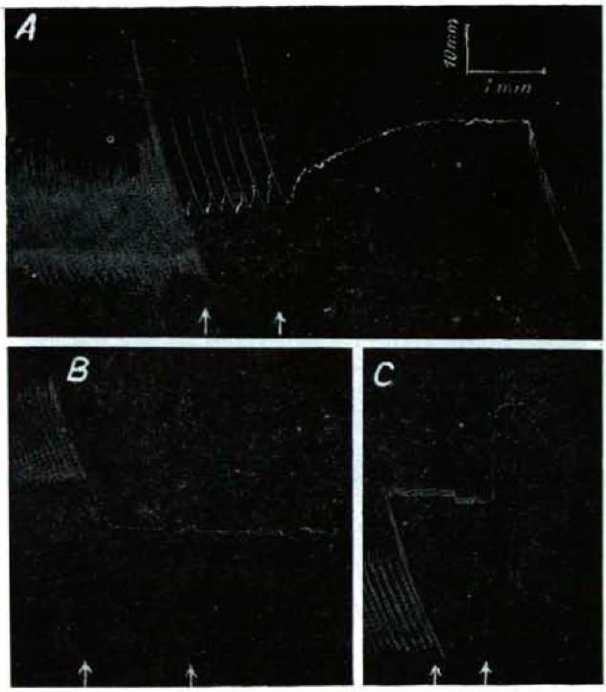

Fig. 5. Effects of strontium, barium, magnesium as calcium-substitutes on the potassium contracture.
A. First arrow: pretreatment in 0.1 mM Ba-Ringer for 30 minutes. Second arrow: 0.1 mM Ba-50 mM K-Ringer.
B. First arrow: pretreatment in 1 mM Mg-Ringer for 20 minutes. Second arrow: 1 mM Mg-50 mM K-Ringer.
C. First arrow: pretreatment in 1 mM Sr-Ringer for 10 minutes. Second arrow: 1 mM Sr-50 mM K-Ringer.

From among the possible calcium-substitutes, we have investigated in detail the effects of the alkaline earth metal ions as these can be considered to be in interaction in several objects with various known calcium effects. The surface membrane binding sites must be common and, according to the investigations of PAPAHADJOPOULOS (1968), HAFEMANN (1969), and others, they are probably phospholipids, and oxigen ligands, or more exactly PO_4^{3-} groups.

In the graph shown in Fig. 2, plotted against extracellular barium concentration, three different affects are presented. It can be seen from the first curve that the contracture rises linearly with the logarithm of the barium concentration (between 1–0.1 mM), according to the data measured at the end of the 30-minute pretreatment (cf. the mechanograms shown in Fig. 4 too). This tonic contracture level till 0.5 mM Ba-Ringer, as seen from the second curve, can be raised to an increasing degree by the 50 mM KCl applied. From 0.5 mM extracellular barium concentration, however, a decrease takes place in the KCl-induced contracture. The third curve shows the difference of the two previous ones, that may be compared with the data obtained for the various calcium concentrations. It can be seen from comparison of the two curves that the potassium contracture till 0.5 mM $(Ca^{2+})e$ or $(Ba^{2+})e$ exhibits a similar slope but in a barium-containing medium the contracture level is more raised. From 0.5 mM, in the Ca-Ringer, a steep rise follows in the contracture as a result of K-depolarization, while in the Ba-Ringer a strong decrease can be observed (Fig. 4).

In the Sr-Ringer a lesser rise in tone takes place as compared with the Ba-Ringer. The height of the contracture obtained in 1 mM Sr-50 mM K-Ringer rather resembles that of barium, and is about 20 per cent of the response measured in 1 mM Ca-50 mM K-Ringer (control).

In 1 mM Mg-Ringer the tone falls and after a pretreatment for 20 minutes the contracture cannot be elicited generally by 50 mM KCl (Fig. 5). We have not investigated the Mg-effect in other relations as it is treated in detail in the paper of BURTON and LOUDON (1972) in connection with calcium antagonism.

It can be seen from the investigations carried out that in the excitation-contraction coupling of the potassium contracture barium and strontium can be substituted partially for calcium. Until 0.5 mM this replacement is very striking, following the sequence of the reciprocal hydrated ion-radius: $Ba \geqslant Sr > Ca \gg Mg$. At a higher concentration, there is only a partial replacement, calcium coming into prominence and the sequence changing to $Ca \gg Ba > Sr \gg Mg$. Both variants belong to the seven sequences actually observed from among the twenty-four possible sequences (WRIGHT and DIAMOND, 1968). To clarify the cause of the change in sequence, further investigations are needed. The reason of the change may be that, on proceeding towards higher concentrations, the independent contracture-eliciting effect of the barium ion prevails more and more, supposing a mechanism that is different from its calcium-substituting part in the potassium contracture and inducing an interaction at the expense of the latter. The sequence based on the reciprocal hydrated ion-radius valid at 0.5 mM extracellular ion concentration resembles the result of PAPPANO (1970), who found the same ionic sequence to be valid in the re-establishment tendency on the action potential of the K-depolarized guinea pig atrial muscle. At the same time, it differs from the data published by FRANK (1962) for the

toe muscle of *Rana pipiens* and by GAINER (1968) for the muscles of the lobster.

For barium or strontium ions to replace calcium in analogous processes, their uptake in the membrane binding sites must be assumed and, owing to the K-depolarization of the membrane, a change is to be expected in the uptake. Experimental investigations were made of the barium ion uptake and the possibility of washing out the isotope taken up. The isotope experiments were carried out under the same conditions as the physiological ones, in 0.3 or 0.1 mM Ba-Ringer with 30 ml total volume (pH = 7.4, 22°C). ^{140}Ba was added to the inactive carrier in a quantity of about 0.6 μCi/ml. The uptake was plotted against time, as a percentage of the incubating solution, referred to 1 mg dry weight. The results are shown in Fig. 6. It can be seen well that till the thirtieth minute, in the period of pre-incubation, in the probably two-compartment systems, the uptake for both concentrations (0.3 and 0.1 mM $BaCl_2$) approximately attained the saturation value. Corresponding to the more increased rise in tone, the uptake is stronger at the higher Ba-concentration (cf. also Figs. 4. C and 5. A). The depolarization elicited by 50 mM KCl increases the ^{140}Ba uptake, corresponding to the stronger contracture, in 0.3 mM Ba-Ringer to a greater extent. In the last section, the decrease in the uptake coincides with a spontaneous decrease in the potassium contracture.

Fig. 7 shows the washing out of ^{140}Ba plotted against the time, in the

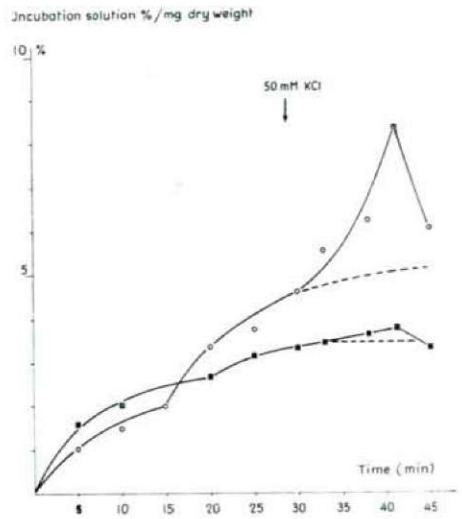

Fig. 6

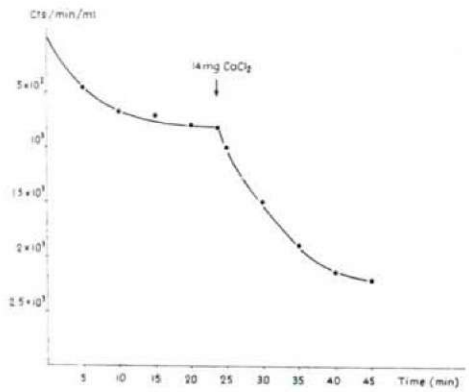

Fig. 7

Fig. 6. Change in the ^{140}Ba uptake of the Helix hearts plotted against time. 0 = in 0.3 mM Ba-Ringer, □ = in 0.1 mM Ba-Ringer. At the point of time marked with an arrow, 50 mM KCl was put into the organ bath. Every point is the mean value of three parallel experiments. The incubating solution contained about 0.6 μCi/ml ^{140}Ba (pH = 7.4, 22 °C).

Fig. 7. ^{140}Ba wash-out curve plotted against time. After uptake for 45 minutes, the washing out began in a Ca-free Ringer. At the point of time marked with an arrow, 14 mg $CaCl_2$ was mixed with 30 ml organ bath, corresponding to 4 mM Ca-Ringer. Every point is the mean value of three parallel experiments (pH = 7.4, 22°C).

Ringer of 0.3 mM $BaCl_2$ content, after an uptake for 45 minutes. Washing out took place in 30 ml Ca-free Ringer, after being rinsed three times for one minute each. At the point of time marked with an arrow 14 mg $CaCl_2$ was added to the Ca-free Ringer, corresponding to 4 mM Ca-Ringer. According to the antagonism of the two ions demonstrated earlier (ERDÉLYI, 1968), the loss

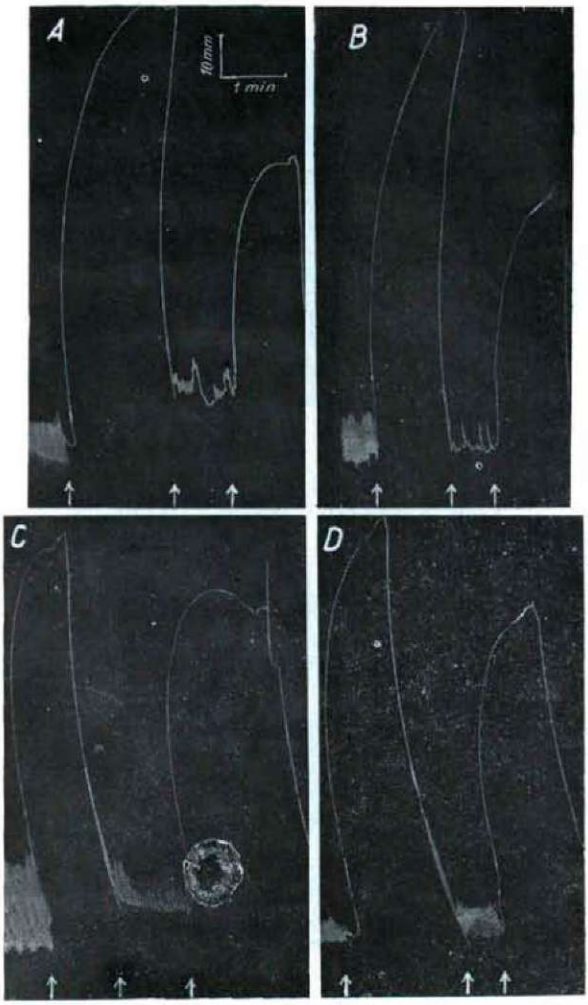

Fig. 8. Effect of change in extracellular lathanum concentration on the potassium contracture. A. First arrow: control contracture in 1 mM Ca-50 mM K-Ringer. Then a 10-minute washing period followed. Second arrow: 5-minute pretreatment in 0.5 mM La-1 mM Ca-Ringer. Third arrow: 0.5 mM La-1 mM Ca-50 mM K-Ringer.
B. First arrow: control contracture, as under „A". Second arrow: 5-minute pretreatment in 0.2 mM La-1 mM Ca-Ringer. Third arrow: 0.2 mM La-1 mM Ca-50 mM K-Ringer.
C. First arrow: control contracture, as under „A". Second arrow: 5-minute pretreatment in 0.02 mM La-1 mM Ca-Ringer. Third arrow: 0.02 mM La-1 mM Ca-50 mM K-Ringer.
D. First arrow: Control contracture, as under „A". Second arrow; 5-minute pretreatment in 0.005 mM La-1 mM Ca-Ringer. Third arrow: 0.005 mM La-1 mM Ca-50 mM K-Ringer.

of ^{140}Ba increases, and the tone in the mechanogram decreases. Parallel with the advance in time of the assumed Ba^{2+}–Ca^{2+} exchange in the binding sites, besides the decrease in tone, the spontaneous activity of the heart is increasingly restored too (Fig. 4, C).

In the further experiments, in 1 mM Ca-50 mM K-Ringer, from 0.005 till 1 mM, the effect of the extracellular lanthanum concentration on the potassium contracture was investigated. It is known that lanthanum, the ionic radius of which is close to that of calcium, and which forms stronger bonds, can be bound to the surface binding sites of the membrane, in this way interacting with calcium in the excitation-contraction coupling (SANBORN and LANGER, 1970; GOODMAN and WEISS, 1971).

Fig. 8 shows the effects of different $(La^{3+})_e$ concentrations in the heart of the edible snail. The first curve is always the control contracture, in 1 mM Ca-50 mM K-Ringer. The second curve shows the contracture obtained with 50 mM KCl, after 10 min washing and 5 min lanthanum-pretreatment in a Ringer of appropriate lanthanum content. In each of the hearts, here too, only the effect of one lanthanum concentration was investigated, in at least three parallel experiments. The data obtained are illustrated graphically in Fig. 9. The Figure shows the change in the contracture as a percentage of the control,

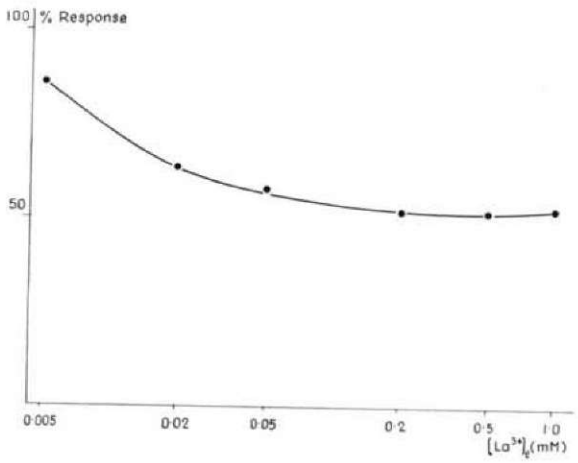

Fig. 9. Effect of the external lanthanum concentration on the contracture elicited in 1 mM Ca-50 mM K-Ringer. The results are expressed as a percentage of the control. Every point is the mean value of three experiments.

plotted against 0.005–1 mM extracellular lanthanum concentration. It can be seen in the graph that an inhibition higher than 50% was not obtained in the contracture elicited by 50 mM KCl, even in the case of the largest lanthanum concentration applied. In spite of the different KCl concentrations, these data show a fairly good agreement with the results of GOODMAN and WEISS (1971) for mammalian vascular smooth muscles and other smooth muscles. The only difference is that, up to 1 mM lanthanum concentration the authors cited observed a stronger inhibition in the objects investigated than we did in the *Helix* heart. According to the Figure, in the *Helix* heart essentially two sections

can be distinguished in the potassium contracture, plotted against the extracellular lanthanum concentration. One of the sections lasts till 0.2 mM lanthanum concentration. Here a considerable decrease in the contracture can be observed. Later, towards higher concentrations the slope of the curve is reduced, the process of inhibition becomes slower and there is no stronger inhibition than 50% even in a domain of higher than 1 mM concentration. It is shown by all this that the *Helix* heart is less sensitive to the influence of this ion than the vertebrate muscles are, and that in this object the lanthanum ion is in direct interaction with only few Ca^{2+}-binding sites.

Discussion

It can be considered as a physiological phenomenon of general validity that if any muscle is put in a medium of excess potassium, after the membrane depolarization a contraction follows, as a function of the extracellular potassium concentration. Similarly to the data published for the vertebrate muscles, this is essentially valid for the various molluscan muscles, too (OZEKI, 1964; NOMURA, 1965; HILL, GREENBERG, IRISAWA, NOMURA, 1970; BURTON and LOUDON, 1972). In bringing about the contraction the extracellular potassium concentration plays a definite part. This is shown by the experiments that analyse the change in potassium contracture as a function of the $(Ca^{2+})_e$ concentration. In this relation there can also be established a considerable resemblanse in the hearts of the animals belonging to various branches. The similarity of the physiological phenomena supposes the possibility of identical mechanisms in the excitation-contraction coupling. In this relation, the regularly arranged sarcotubular system, demonstrated from the heart of the *Helix pomatia*, may play the intermediary part which must be assumed on the basis of the investigations carried out on vertebrate muscles (Physiol. Symp. 1965, PHILIPOTT and GOLDSTEIN, 1967; HOFMANN, 1969; JOHNSON and LIEBERMAN, 1971; and others). It seems that in bringing about the contracture, that is in releasing the intracellular ionic calcium, apart from the sarcotubular system, the Ca^{2+}-binding sites localized on the membrane surface also play a considerable part. It has not yet been cleared up completely as to how these two mechanisms co-operate. At any rate it is probable that a possibility of activating and inactivating mechanisms must be supposed here too (BEELER and REUTER, 1970, and others). Taking into consideration the nature of the surface Ca^{2+}-binding sites or stores, the difference established between the hearts of frogs and snails, as well as in the case of the lobster muscle, in the relation of the antagonistic actions of Ca-Na, Ca-Mg, or Ca-K in the competition for the identical binding sites, is noteworthy (NOMURA, 1965; GAINER, 1968; BURTON and LOUDON, 1972).

It is shown by our experiments on the potassium contracture that these important Ca^{2+}-binding sites or stores may interact with each of the alkaline earth metal ions. But the connection between the alkaline earth metal ion and the surface membrane binding site does not induce favourable conditions to an equal extent for bringing about the excitation-contraction coupling. In this relation, both the cation configuration and the force of the binding site created may be a determining factor. With each alkaline earth metal ion an interaction can take place that is similar to that with the calcium ion. This conclusion can

be drawn from the isotope experiments with the barium ion, in so far as the ^{140}Ba uptake was increased by the K-depolarization while the washing out of the isotope taken up was accelerated by the increased extracellular calcium concentration, corresponding to the antagonism of the two ions. Different concentrations of the various alkaline earth metal ions must be regarded as optimum from the point of view of the function of the excitation-contraction coupling. This may be concluded from the change in ion sequence taken as a function of the extracellular concentration. Up to 0.5 mM, the ionic sequence based on the reciprocal hydrated ion radius prevailed, that is $Ba \geqslant Sr > Ca \gg Mg$. From 0.5 mM this changed in favour of the calcium ion, and the sequence $Ca \gg Ba \geqslant Sr \gg Mg$ prevailed, with a strong decrease of the calcium-substituting role of the other alkaline earth metal ions. As several effects of the alkaline earth metal ions on biological systems are known including the action potential, the influence exerted on the membrane permeability, membrane resistance, metabolism, etc., the phenomenon produced is more complicated than the interaction brought about with the surface Ca^{2+}-binding sites or stores. It seems to be probable that the lanthanum ion is associated with essentially the same calcium-sensitive membrane binding sites or stores through which the extracellular calcium ion exerts its regulative effect upon the excitation-contraction coupling (GOODMAN and WEISS, 1971). By means of its stronger binding, the trivalent lanthanum ion may fix these membrane binding sites in a configuration established so firmly that they cannot completely fulfil their physiological part in the mechanism of releasing the intracellular calcium ion. This phenomenon may be brought into connection with the effect of the lanthanum ion in inhibiting the potassium contracture, that is similarly to a certain extent a function of the extracellular lanthanum ion concentration. It seems that the mammalian muscles are more sensitive to lanthanum, for the lanthanum ion concentration that resulted in a 100% inhibition of the potassium contracture in the mammalian muscle, could not inhibit the contractility of the heart by more than 50% in the *Helix* heart. All this indicates the supposed differences as regards the density of the binding sites and the sensitivity of the binding site populations.

Summary

A study was made of the effect of the extracellular potassium concentration on the *Helix* heart contracture, and the calcium-dependence of the phenomenon. As regards the potassium contracture, the calcium-substituting capacity of the alkaline earth metal ions was examined. In this respect, a concentration-dependence was ascertained in the case of barium and strontium. Up to 0.5 mM, bot alkaline earth metal ions were found to be effective calcium-substitutes. At 1 mM, however, only a partial calcium replacement of a strongly reduced degree is found. On the basis of data measured at a 0.5 mM alkaline earth metal ion concentration, the effect exerted on the potassium contracture follows the sequence of the reciprocal hydrated ion radius: $Ba \geqslant Sr > Ca \gg Mg$. At 1 mM this sequence changes, the calcium ion coming strongly into prominence: $Ca \gg Ba > Sr \gg Mg$. In the excitation-contraction coupling the magnesium ion is practically unable to play the part of a calcium substitute.

The barium effect is probably associated with the surface membrane Ca^{2+}-binding sites. This can be concluded from the isotope experiments in so far as the K-depolarization, like calcium, can increase the ^{140}Ba uptake, while the washing out of the isotope taken up is considerably increased by raising the extracellular calcium concentration, corresponding to the antagonism of the two ions.

The lanthanum ion (0.005–1 mM) could inhibit the potassium contracture, in an interaction with the surface membrane Ca^{2+}-binding sites or stores, but to a lower degree than expected, up to a maximum 50%. This points to the lower sensitivity of the Helix heart, as compared to vertebrate muscles.

References

BEELER JR., G. W., REUTER, H. (1970): The relation between mebrane potential, mebrane currents and activation of contraction in ventricular myocardial fibres. — J. Physiol. *207*, 211—229.

BURTON, R. F., LOUDON, J. R. (1972): The antagonistic actions of calcium and magnesium on the superfused ventricle of the snail *Helix pomatia*. — J. Physiol. *220*, 363—381.

ERDÉLYI, L. (1968): Examination of barium-calcium antagonism in hearts and smooth-muscle organs of the edible snail (*Helix pomatia* L.). — Acta Biol. Acad. Sci. Hung. *19*, 11—22.

ERDÉLYI, L. (1971): Effects of ion milieu and barium ions on the ventricular electrocardiogram of the edible snail (*Helix pomatia* L.). — Acta Biol. Szeged *17*, 171—184.

ERDÉLYI, L., HALÁSZ, N. (1972): Electron-microscopical observations on the auricle of snail heart (*Helix pomatia* L.) with special regard to the structure of granulated cells. — Acta Biol. Szeged *18*, 253—267.

FRANK, G. B. (1962): Utilization of bound calcium in the action of caffeine and certain multivalent cations on skeletal muscle. — J. Physiol. *163*, 254—268.

GAINER, H. (1968): The role of calcium in excitation-contraction coupling of lobster muscle. — J. Gen. Physiol. *52*, 88—110.

GOODMAN, F. R., WEISS, G. B. (1971): Dissociation by lanthanum of smooth muscle responses to potassium and acetylcholine. — Amer. J. Physiol. *220*, 759—766.

GOODMAN, F. R., WEISS, G. B. (1971): Effects of lanthanum on Ca^{45} movements and on contratctions induced by norepinephrine, histamine and potassium in vascular smooth muscle. — J. Pharm. Exp. Ther. *177*, 415—425.

HAFEMANN, D. R. (1969): Effects of metal ions on action potentials of lobster giant axons. — Comp. Biochem. Physiol. *29*, 1149—1161.

HILL, R. B., GREENBERG, M. J., IRISAWA, H., NOMURA, H. (1970): Electromechanical coupling in a molluscan muscle the radula protractor of *Busycon canaliculatum*. — J. Exp. Zool. — *174*, 331—348.

HOFMANN, W. W. (1969): Membrane potential and muscle function. — Ann. Rev. Med. *20*, 101—130.

IRISAWA, H., KOBAYASHI, M. (1964): On spontaneous activities of oyster myocardium caused by several inorganic ions in sucrose solution. — Jap. J. Physiol. *14*, 165—176.

JOHNSON, E. A., LIEBERMAN, M. (1971): Heart excitation and contraction. — Ann. Rev. Physiol. *33*, 479—532.

LAMB, J. F., MCGUIGAIN, J. A. S. (1966): Contractures in a superfused frog's ventricle. — J. Physiol. *186*, 261—283.

LÜTTGAU, H. C., NIEDERGERKE, R. (1958): The antagonism between Ca and Na ions on the frog's heart. — J. Physiol. *143*, 486—505.

NIEDERGERKE, R. (1956): The potassium chloride contracture of the heart and its modification by calcium. — J. Physiol. *134*, 584—599.

NIEDERGERKE, R. (1963): Movements of Ca in frog heart ventricles at rest and during contractures. — J. Physiol. *167*, 515—550.

NOMURA, H. (1965): Potassium contracture and its modification by cations in the heart muscle of the marine mollusc, *Dolabella auricula*. — Jap. J. Physiol. *15*, 253—269.

NORTH, R. J. (1963): The fine structure of the myofibers in the heart of the snail *Helix aspersa*. — J. Ultrastruct. Res. *8*, 206—218.

OZEKI, M. (1964): Effect of calcium and sodium ions on potassium induced contracture and twitch tension development of the retractor pharynx muscle of a snail. — Jap. J. Physiol. *14*, 155—164.

PAPAHADJOPOULOS, D. (1968): Surface properties of acidic phospholipids: interaction of monolayers and hydrated liquid crystals with uni- and bi-valent metal ions. — Biochem. Biophys. Acta. *163*, 240—254.

PAPPANO, A. J. (1970): Calcium-dependent action potentials produced by catecholamines in guinea pig atrial muscle fibers depolarized by potassium. — Circ. Res, *27*, 379—390.

PHILPOTT, CH. W., GOLDSTEIN, M. A. (1967): Sarcoplasmic reticulum of striated muscle: localization of potential calcium binding sites. — Science (Washington) *155*, 1019—1021.

PHYSIOL. SYMP. ATLANTIC CITY, NEW JERSEY. (1965): Excitation-contraction coupling in striated muscle. — Fed. Proc. *24*, 1112—1152.

SANBORN, W. G., LANGER, G. A. (1970): Specific uncoupling of excitation and contraction in mammalian cardiac tissue by lanthanum. — J. Gen. Physiol. *56*, 191—217.

SCHLOTE, F. W. (1964): Die Muskulatur von *Helix pomatia* und ihre Innervation. — Proc. First. — Europ. Malac. Congr. 113—151.

WEISS, G. B. (1970): On the site of action of lanthanum in frog sartorius muscle. — J. Pharmacol. Exptl. Therap. *174*, 517—526.

WILBRANDT, W., KOLLER, H. (1948): Die Calcium Wirkung am Froscherzen als Funktion des Ionengleichgewichts zwischen Zellmembran und Umgebung. — Helv. Physiol. pharmacol. Acta. *6*, 208—221.

WRIGHT, E. M., DIAMOND, J. M. (1968): Effects of pH and polyvalent cations on the selective permeability of gall-bladder epithelium to monovalent ions. — Biochem. Biophys. Acta. *163*, 57—74.

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INHIBITORY SYNAPSES ON THE PERIKARYONS OF MITRAL, TUFTED AND GRANULAR CELLS OF THE RAT OLFACTORY BULB

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Introduction

GRAY (1962) first suggested the possiblity of distinguishing the excitatory or inhibitory natures of neural connections on the basis of the ultrastructural characteristics of the synapses. The combined aldehyde-osmium fixation method (UCHIZONO, 1965) soon became one of the routine electron-microscopic preparatory procedures, and provided a new possibility for the distinction on the basis of the difference of the vesicle forms of the presynaptic element. After further work, doubt arose as to the application of the above possibilities, but according to present knowledge certain central and peripheral nervous system synapses can be well charaterized fine-structurally.

This paper wishes to draw attention to synapses observed on the soma of the neurones of the rat olfactory bulb; the inhibitory nature of these is suggested on the basis of fine-structural criteria and earlier physiological observations.

Materials and Methods

14 fully developed (4—6 months old) albino rats were used in the study. The animals were anaesthetized with Nembutal and perfused for 30 minutes. The olfactory bulb was removed, segmented with orientation, and submitted to immersion fixation in a solution prepared according to KARNOVSKY (1956). This was followed by post-fixation for 30 minutes in a buffered 1% OsO_4 solution (MILLONIG, 1962), dehydration in the usual manner, and embedding in araldite (Durcupan, Fluka). Sections were prepared on a Tesla ultrotome. Semithin (0.5—0.7 μ) samples were prepared simultaneously with the thin sections, and the electron-microscope studies were checked continually with the help of these. Photographs were taken on Tesla BS 242 D and JEOL 100 B electron-microscopes.

Results

The fine-structural characteristics of the structure of the rat olfactory bulb have aroused the interest of many research workers. One of the relevant papers (ANDRES, 1965) presents a detailed analysis of the structure of olfactory bulb, the neurones and glia cells of its layers, and some of its synapses. It is known from the above work too that on proceeding inwards one encounters in the olfactory bulb a layer of fila olfactoria, and the glomerular, outer plexiform,

mitral, inner plexiform and granular layers. Our work deals with the synaptic relations of the mitral, tufted and granular cells, and with the three characteristic neurones of these layers, with particular regard to the synapses on the soma.

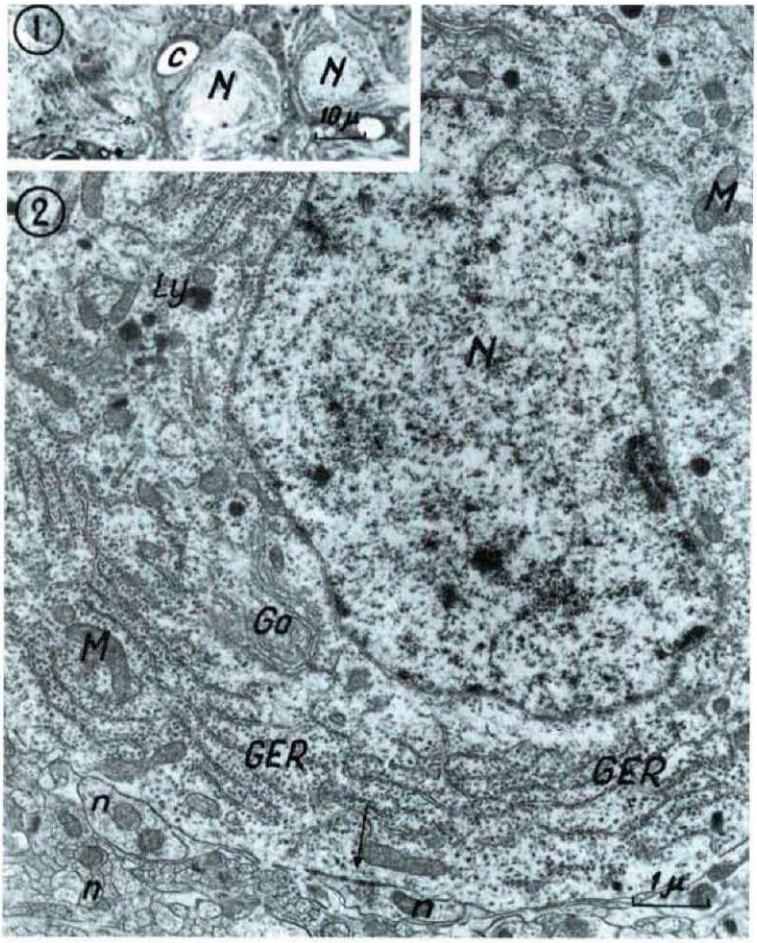
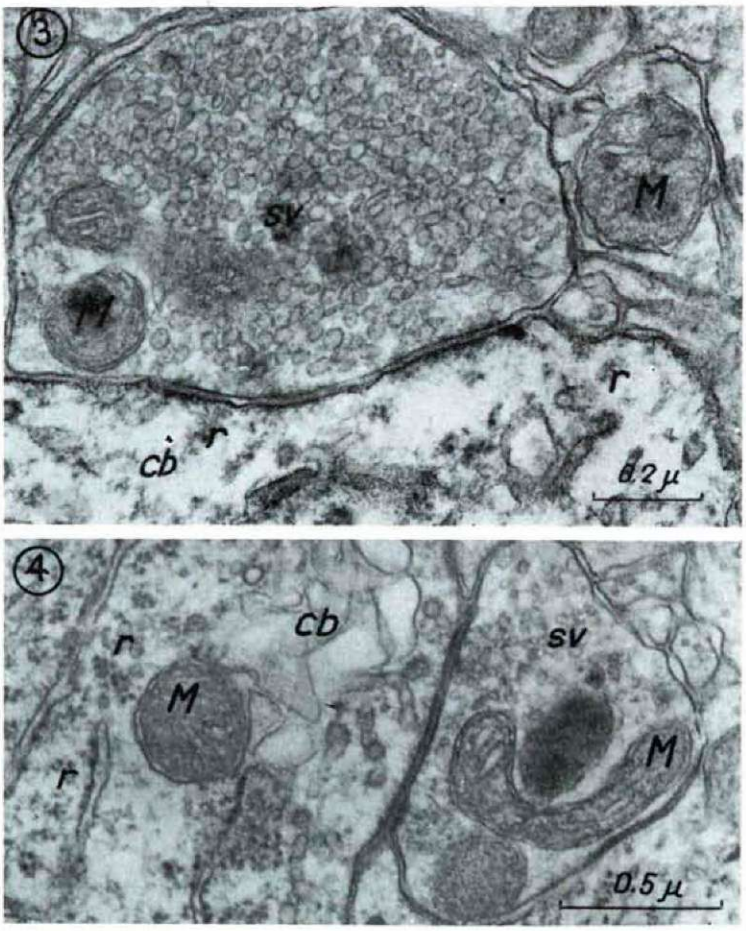


Fig. 1. Mitral cells on semi-thin section (toluidine blue staining). N = nucleus, c = capillary.
Fig. 2. Electron-microscope picture of mitral cell. N = nucleus, Ly = lysosome, M = mitochondrium, GER = granulated endoplasmic reticulum, Go = Golgi apparatus, n = nerve fibre. The arrow indicates the synapse.

The mitral cells (Figs. 1–2) are arranged in a single row of cells; their axons are grouped in the lateral olfactory tract; branching in the region of the glomerulus olfactorius, their main dendrites receive the excitatory impulses of the fila olfactoria, while their secondary dendrites form numerous synapses with the processes of the granular cells in the str. plexiforme externum. The perikaryon is richly provided with cytoplasmatic organelles (Fig. 2), and nerve

fibres frequently lie close enough to its enormous external surface to be suitable for the formation of synaptic connections (Figs. 2–4). In the connection of the perikaryon and its environment one can observe a synapse from the perikaryon towards the nerve fibre (Gray-II type), and also connections of the nerve fibres, presumably polarized in the direction of the perikaryon; the two can sometimes be observed side by side in the form of a reciprocal synapse. We shall deal here only with the synaptic systems polarized towards the soma. These synapses (Figs. 2–4) are characterized by the fact that the aldehyde-osmium fixing method used reveals ovoid vesicles in them. The density of the vesicles is variable: they sometimes fill the whole of the fibre (Fig. 3), while at other times they can be observed only sporadically (Figs. 2 and 4). Round and ovoid vesicles appear among them in various proportions. The exact spheroidal-ovoid ratio can be determined only with a goniometer. Neverthelles,



Figs. 3—4. Grey-II. type synapses on soma of mitral cells. M = mitochondrium, sv = synaptic vesicles, cb = mitral perikaryon, r = ribosomes.

the size of the ovoid vesicles is always less than that of the round vesicles, and independently of the form they can be identified with certainty in the given plane.

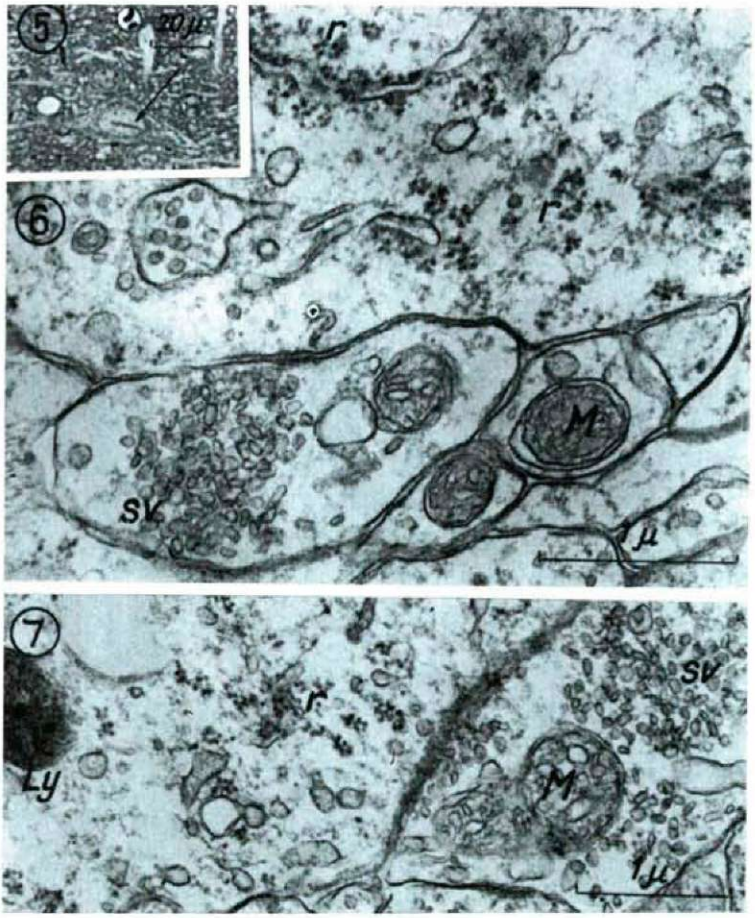


Fig. 5. Tufted cell on semi-thin section. The arrow indicates the nucleus of the tufted cell. Figs. 6—7. Synapses on body of tufted cells. M = mitochondrium, sv = synaptic vesicles, Ly = lysosome, r = ribosomes.

The tufted cells (Fig. 5) are found in the region of the str. plexiforme externum, and are often difficult to distinguish from other cell types. The light- and electron-microscope studies carried out in parallel provide a good possibility for the elemination of this source of errer. The cytoplasm/nucleus ratio for the tufted cell is less than that for the mitral cells, and the size of the cells too is variable. Many nerve fibres reach at the perikaryon, similarly to that of the mitral cells, and here too synapses polarized towards the perikaryon are frequently found (Figs. 6–7), and in places may be considered as reciprocal. The vesicle density of the nerve fibres too is similar to that of the fibres connected

to the mitral cells discussed above, but at the same time a greater number of the vesicles exhibit a flattened form. The position of the vesicles varies: they can be observed rarely in the vicinity of the synaptic thickening (Fig. 7), but they never come into such an intimate connection with the presynaptic membrane as usual in the Gray-I type synapses.

The granular cells are small neurones arranged densely beside each other; it appears from a light-microscopic photograph of a semi-thin section (Fig. 8) as if the cell nuclei were in contact with each other. It turns out from electron-microscope photographs that the cell nuclei (Figs. 9–12) are surrounded by only a very thin cytoplasmic border, the thickness of which is sometimes less than 0.2 μ. In this thin cytoplasm only very few organelles can be seen. Nor is

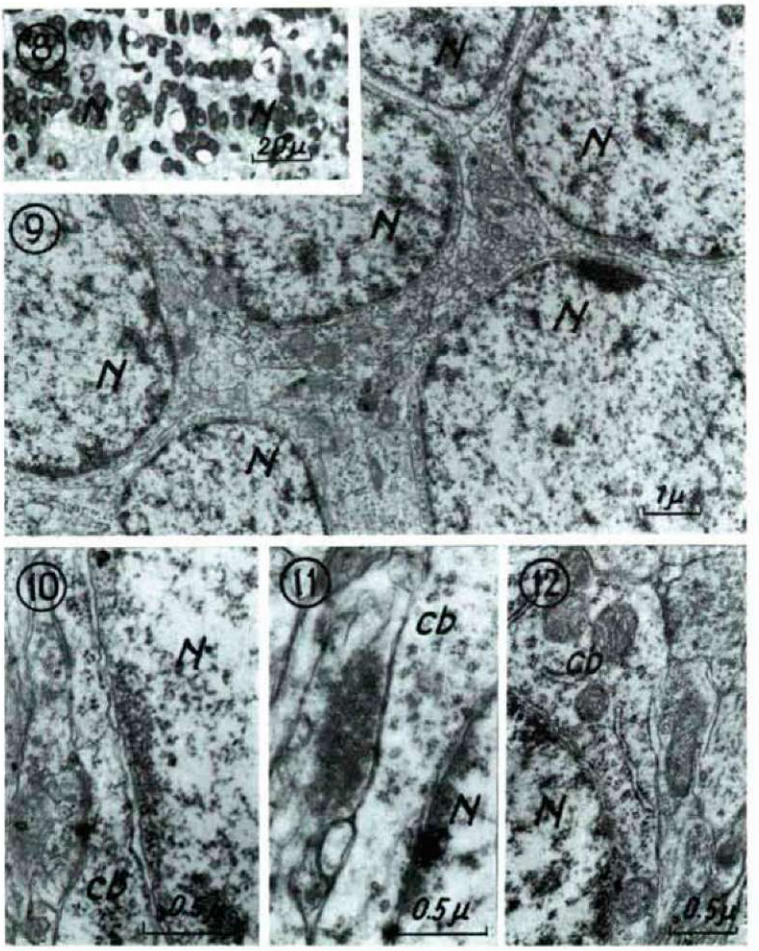


Fig. 8. Granular cells on section stained with toluidine blue.
Fig. 9. Electron-microscope picture of granular cells.
Fig. 10—12. Synapses on perikaryon of granular cells. N = nucleus, cb = granular perikaryon.

there an abundance of granular cells in the synapses. The observed synapses were always found to be unidirectional, that is to be polarized from the nerve fibre towards the cytoplasm of the granular cell. The vesicle density was variable, while the pre- and postsynaptic membranes were thickened to the same extents. Based on their size and form, the vesicles can be classified in the ovoid category.

Discussion

As mentioned in the introduction, the aim of this work was to characterize the synapses observed on the soma of some neurones of the rat olfactory bulb, on the basis of morphological criteria and earlier physiological studies.

Of the different fine-structural criteria of the synapses, attention was drawn by GRAY (1962) primarily to the different natures of the synaptic thickening, when he found that the synapse types reported three years previously (GRAY, 1959) in the cat spinal cord could be correlated with the excitatory or inhibitory natures of these same synapses. This assumption was supported by the results of ECCLES (1964). The characteristics of the membrane specialization were also observed by COLONNIER (1968). The introduction and application of the aldehyde-osmium fixation method permitted a generally consistent distinction between the forms of the synaptic vesicles (UCHIZONO, 1965, 1966; BODIAN, 1966; WALBERG, 1966). Nevertheless, a distinction based on the different forms of the vesicles can not be the only basis for the structural classification of a synapse: a number of papers have drawn attention to this. Thus, relations have been found between vesicle form and age (LARRAMENDI et al., 1967), and between form and osmolality conditions (BODIAN, 1970; VALDIVIA, 1971); as regards certain synapses, however, the relations between ovoid vesicle and inhibition, and spherical vesicle and excitation is dubious (PAPPAS, 1966; PAPPAS and BENNETT, 1966; MUGNAINI, 1970). These examples emphasize the importance of taking into account not only the vesicle form, but also the type of synaptic thickening; as proved by recently published correlations, the two characteristics together can lead to a correct classification with a fair degree of certainty.

PRICE (1968) characterizes the reciprocal dendrodendritic synapses of the rat olfactory bulb in agreement with electrophysiological measurements, and characterizes the excitatory synapsing region by the joint occurrence of asymmetric thickening and round vesicles, and the inhibitory synapsing region by symmetric synaptic mebrane thickening and flattened synaptic vesicles. After aldehyde-osmium fixation, WESTRUM (1969) generally found flattened synaptic vesicles in the Gray-II type synapses of the rat prepyriform cortex. In an earlier publication (HALÁSZ and CSILLIK, 1969), we too reported a similarly good correlation for the rat cerebellum region, while PINCHING (1970) found a connection between the excitatory and inhibitory synapses and their structures in the synapses of the glomerular layer of the rat olfactory bulb. In support of the previous correlations, we have also found the grouping of the vesicles to be characteristic of excitatory and inhibitory, in the two types of synapses (HALÁSZ, in press). According to our observations, which are supported by the present work, the ovoid vesicle form and symmetric synaptic thickening are

not accompanied by the characteristic grouping of the synaptic vesicles beside the presynaptic membrane, as observed in the Gray-I type synapses.

A number of papers discuss the synaptic relations of the mitral, tufted and granular cells of the olfactory bulb at the light- and electron-microscope levels. According to CAJAL (1911), the tufted cell axon may terminate on the perikaryons of the mitral cells. The observation of a reciprocal synapse in the same region is reported by HIRATA (1964) and ANDRES (1965); PRICE and POWELL (1970) also observed synapses on the soma of the mitral cell. According to WILLEY (1969) all of the synapses to be found on the mitral cell are reciprocal; similar conditions can be concluded from our earlier studies (HALÁSZ, in press), but of these only the nerve fibre → mitral cell polarization is dealt with in the present work. In addition to the excitatory impulses picked up by the main dendrite on the mitral cells, an inhibition too is displayed; but the means of transmission of this inhibition to the mitral cell is debatable. The existence of the inhibition is proved with regard to the granular dendrite → mitral secondary dendrite in the reciprocal dendrondritic synapses of the str. plexiforme externum (SHEPHERD, 1963). it is probable that at the same time the mitral cell receives an inhibition on the perikaryon too. The synapsing nerve fibres found here by us can be characterized by the ovoid vesicles, the symmetric synaptic membrane thickening and the scattered arrangement of the vesicles. Based on the above observations and the results provided by the physiology, we consider these nerve fibres to be inhibitory. This nerve fibre may originate from an interneurone, whether that be the granular cell or one of the short-axon cells (PRICE and POWELL, 1970).

The synaptic relations of the tufted cells are less well elucidated. Some hold the view that the tufted cell is to be interpreted as a secondary neurone similarly to the mitral cell, on the grounds that it may be excited in a similar way to the mitral cell (NICOLL, 1972). An inhibitory effect has also been demonstrated on their secondary dendrites (RALL et al., 1966; WESTECKER, 1970. According to other conclusions, the tufted cell is a modified periglomerular cell, and thus an interneurone, as supported by the studies of HINDS (1970). This latter assumption does not exclude the possibility that the soma may receive inhibitory impulses, as occurs in the case of other interneurones in the glomerular layer of the olfactory bulb (PINCHING, 1970). At the same time, the structural characteristics seem clear-cut. On the above basis, the observed synapses are classified as of an inhibitory nature.

The positions of the fibres of the granular cells were determined by CAJAL (1911). Accounts of the soma synapses have also been published by HIRATA (1964) and ANDRES (1965). HIRATA (1964) referred to the possibility that this connection might be inhibitory. It does not emerge from the OsO_4-fixed material of ANDRES (1965) which conception may be supported on the basis of the visecle form of the synapse in question. In the present work it is considered that the characteristics of the inhibitory synapses are disclosed on the fibre synapsing with the granular perikaryon. The fibre may be a part of an interneuronal system similar to that found by PINCHING (1970) in the glomerular layer of the olfactory bulb. Electrophysiological studies are called for to clarify the further characteristics of these neuronal cycles, as has already been done with regard to the primary neurones of the olfactory bulb.

Summary

The synaptological relations of the mitral, tufted and granular cells of the rat olfactory bulb are dealt with. Based on the synaptic thickening, and the form and position of the vesicles, it is found that the nerve fibres synapsing with the perikaryons of the above cells may be of an inhibitory nature. This assumption is discussed in connection with the relevant electrophysiological results.

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References

ANDRES, K. H. (1965): Der Feinbau des Bulbus olfactorius der Ratte unter besonderer Berücksichtigung der synaptischen Verbindungen. — Z. Zellforsch. *65*, 530—565.

BODIAN, D. (1966): Electron microscopy: Two major synaptic types on spinal motoneurons. — Science *151*, 1093—1094.

BODIAN, D. (1970): An electron microscopic characterization of classes of synaptic vesicles by means of controlled aldehyde fixation. — J. Cell Biol. *44*, 115—124.

CAJAL, RAMÓN, Y. (1911): Histologie du Systéme Nerveux de l'Homme et des Vertébres. Maloine, Paris, Vol. II. (re-issued 1952 and 1955, Consejo Superior de Investigationes Cientificas, Madrid).

COLONNIER, M. (1968): Synaptic patterns of different cell types in the different laminae of the cat visual cortex. An electron microscope study. — Brain Res. *9*, 268—287.

DUNCAN, D., MORALES, R. and BENIGNUS, V. A. (1970): Shapes and sizes of synaptic vesicles in the cerebellum of the syrian hamster cortex and deep nuclei. — Anat. Rec. *168*, 1—8.

ECCLES, J. C. (1964): The physiology of synapses. Acad. Press. New York.

GRAY, E. G. (1959): Axosomatic and axodentritic synapses of the cerebral cortex: an electron microscope study. — J. Anat. (London) *93*, 420—433.

GRAY, E. G. (1962): A morphological basis for presynaptic inhibition? — Nature *193*, 82—83.

HALÁSZ, N. (in press): Aldehyde-osmium fixation of the rat olfactory bulb: correlation between synaptic patterns and nature of the transmission. — Acta Anat.

HALÁSZ, N. and CSILLIK, B. (1969): Experimental electron microscopic studies on excitatory and inhibitory synapses in the cerebellar cortex of the rat. — Exp. Brain Res. *9*, 73—82.

HINDS, J. W. (1970): Reciprocal and serial dendrodendritic synapses in the glomerulat layer of the rat olfactory bulb. — Brain Res. *17*, 530—534.

HIRATA, Y. (1964): Some observations on the fine structure of the synapses in the olfactory bulb of the mouse, with particular reference to the atypical synaptic configurations. — Arch. histol. jap. *24*, 292—302.

KARNOVSKY, M. J. (1965): A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. — J. Cell Biol. *27*, 137a—138a.

LARRAMENDI, R. M. H., FICKENSCHER, I. and LEMKEY—JOHNSTON, N. (1967): Synaptic vesicles of inhibitory and excitatory terminals in the cerebellum. — Science *156*, 967—969.

MILLONIG, G. (1961): Advantages of a phosphate buffer for OsO_4 solutions in fixation. — J. appl. Physics *32*, 1637.

MUGNAINI, E. (1970): Neurones, as synaptic targets. In: Excitatory synaptic mechanisms. Eds. P. ANDERSEN and J. K. S. JANSEN, Universitetsforlaget Oslo, etc.

NICOLL, R. A. (1972): Olfactory nerves and their excitatory action in the olfactory bulb. — Exp. Brain Res. *14*, 185—197.

PAPPAS, G. D. (1966): Electron microscopy of neuronal junctions involved in transmission in

the central nervous system. In: Nerve as a tissue. Eds. K. RODAHL and B. ISSEKUTZ. Harper and Row, New York.

PAPPAS, G. D. and BENNETT, M. V. L. (1966): The fine structure of vesicles associated with excitatory and inhibitory junctions. — Biol. Bull. *131*, 381.

PINCHING, A. J. (1970): Synaptic connections in the glomerular layer of the olfactory bulb. — J. Physiol. *210*, 14P—15P.

PRICE, J. L. (1968): The synaptic vesicles of the reciprocal synapse of the olfactory bulb. — Brain Res. *11*, 697—700.

PRICE, J. L. and POWELL, T. P. S. (1970): The mitral and short axon cells of the olfactory bulb. — J. Cell Sci. *7*, 631—651.

RALL, W., SHEPHERD, G. M., REESE, T. S. and BRIGHTMAN, M. (1966): Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. — Exptl. Neurol. *14*, 44—56.

SHEPHERD, G. M. (1963): Neuronal systems controlling mitral cell excitability. — J. Physiol. *168*, 101—117.

UCHIZONO, K. (1965): Characteristic of excitatory and inhibitory synapses in the central nervous system. — Nature *207*, 642—643.

UCHIZONO, K. (1966): Excitatory and inhibitory synapses in the cat spinal cord. — Jap. J. Physiol. *16*, 570—575.

VALDIVIA, O. (1971): Methods of fixation and the morphology of synaptic vesicles. — J. Comp. Neurol. *162*, 257—263.

WALBERG, F. (1966): Elongated vesicles in terminal boutons of the central nervous system, a result of aldehyde fixation. — Acta Anat. *65*, 224.

WESTECKER, M. E. (1970): Excitatory and inhibitory interactions in the olfactory bulb involving dendrodendritic synapses between mitral cells and granular cells. — Pflügers Arch. *317*, 173—186.

WESTRUM, L. E. (1969): Electron microscopy of degeneration in the olfactory tract and plexiform layer of the prepyriform cortex of the rat. — Z. Zellforsch *98*, 157—187.

WILLEY, T. J. (1969) The fine structure of reciprocal synapses in the cat olfactory bulb. — J. Cell Biol. *43*, 158a.

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MACROCEPHALIC AND „AVAR PERIOD" MONGOLID ANTHROPOLOGICAL FINDS FROM WOIWODINA

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On the occasion of a study-tour in Jugoslavia in 1971 I had an opportunity for having a look of the anthropological materials of the museums in Woiwodina. In spite of the low number of the finds I managed to investigate some skeletons being of interest from the point of view of physical anthropology. In this short account I should like to describe three artificially deformed (macrocephalic) crania and two finds of „Avar-period" Mongolid type.

Description of the finds

In the collection of the Town Museum in Subotica there are two macrocephalic crania having been rescued by the archeologist L. SZEKERES. There are not any reliable data to determine their archeological ages. We shall give a detailed description of them as follows.

Find-spot: Subotica-Sándor Co-operative Home.

Date of discovery is probably 1950.

Cranium in very good state of preservation, age of death about 29 years. On the basis of the value −1.3 of the sexual coefficient (FARKAS–LENGYEL–MARCSIK, 1972) the cranium represents a hyperfeminine character, according to what we determined it as a female.

On the basis of absolute sizes the cranium is short (Fig. 1), narrow, high, oligencephalic, the forehead is narrow, the whole face and the upper face are high, mesognathous. According to the indices (Table 1) it is brachycranic, hypsicranic, acrocranic, metriometopic, hyperleptoprosopic, leptene, mesoconch, mesorrhine, mesostaphyline.

There is ossa Wormiana in the sut. lambdoidea. Though the aberration of the teeth has started, M_3 has developped only in the upper set of teeth. The upper link I_1 can be considered – in spite of the aberration – as shovel-shaped. The nasal bones are wide and they reach up high in the frontal bone. The cranium is plagiocephalic at the occipital region, as well as at the right pars lambdica and pars obelica of the sut. lambdoidea. At both sides from the middle line of the os frontale the bone is flat, and next to the sut. coronalis there is a well perceptible hollow in the occipital direction. At the occlusion of the teeth-curves opisthodontia can be observed.

The g–l length is 147 mm, the basion-antibasion distance is 144 mm, the value of the index of deformation is 197.96, which represents – according

to the scheme of Oetteking and Ginzburg–Žirov (LIPTÁK, 1961) – a macrocranic, that is moderately deformed cranium. On the basis of the basion-antibasion absolute size – according to the scheme of Ginzburg – the cranium is greatly deformed. Considering that the latest value, however, depends also on the taxonomical characteristics of the cranium, we think the conclusion made on the basis of the index is more acceptable.

Fig. 1. Macrocephalic cranium of a female (Subotica—Sándor Co-operative Home)

Drawing conclusions from the morphological characteristics of the cranium, the deformation must have been a circular one having done by pressing down the frontal bone and the occipital bone with the aid of a flat object pushed a little bit to the right, then tying them up, but a binding must have been certainly also behind the sut. coronalis.

Table 1. Metrical and morphological characters of the finds

No. of measurements (Martin)	Subotica		Ada	Bačka Topola	
	Sándor Co-op. Home	Hussar-barrack		1.	2.
1.	154	153	171	187	174
1c.	143	152	162	175	167
5.	96	—	—	99	101
8.	127	140	146	147	141
9.	84	78	86	99	91
17.	138	—	—	137	128
20.	118	—	—	118	113
32a.	49	—	—	51	50
38.	1050	—	—	1467	1265
40.	92	—	—	102	101
45.	121	—	—	146	129
46.	96	72	—	110	109
47.	118	—	—	131	114
48.	72	—	—	78	73
51.	39	—	—	38	37
52.	32	33	—	32	32
54.	26	18	—	31	29
55.	53	39	—	51	52
62.	46	32	—	46	45
63.	39	30	—	42	42
65.	114	—	—	136	120
66.	96	—	—	103	97
69.	31	—	—	42	30
70.	58	—	—	72	62
71.	31	—	—	38	37
72.	81	—	—	81	85
8 : 1	82.5	91.5	85.4	78.6	81.0
17 : 1	89.6	—	—	73.3	73.6
17 : 8	108.7	—	—	93.2	90.8
9 : 8	66.1	—	58.9	67.4	64.5
47 : 45	97.5	—	—	89.7	88.4
48 : 45	59.5	—	—	53.4	56.6
52 : 51	80.1	—	—	84.2	86.5
54 : 55	49.1	46.2	—	60.8	55.8
63 : 62	94.8	93.8	—	91.3	93.3
N. vert.	—	—	—	Ov.	Pent.
Glabella	1	1	2	3	1
Sp. nas. ant.	2	3	—	1	1
Pr. occ. ext.	1	0	2	2	1
Progn. alv.	2	1	—	3	3
Fossa can.	3	1	—	2	1
Age	29	6	75	60	29
Sexualis.	— 1.3	—	+ 1.5	+ 1.4	— 1.0

The nasal bones reaching far up, the high corpus mandibulae, the shovel-shaped for-teeth all represent at least europo-mongolid characteristics from the point of view of race-diagnistics (LIPTÁK, 1971).

Find-spot: Subotica-Hussarbarrack.

The find came to the light in 1963 and it is to be dated from the Avar period. Also this cranium is deposited in the Town Museum in Subotica.

Fragmentary calvarium in good state of preservation. As among the permanent teeth the eruption of M_1 has not been completed, the age of death concerning this find can be estimated at 6 years. Sex cannot be determined with absolute certainty.

On the basis of the indices the cranium (Fig. 2) is hyperbrachycranic and stenometopic.

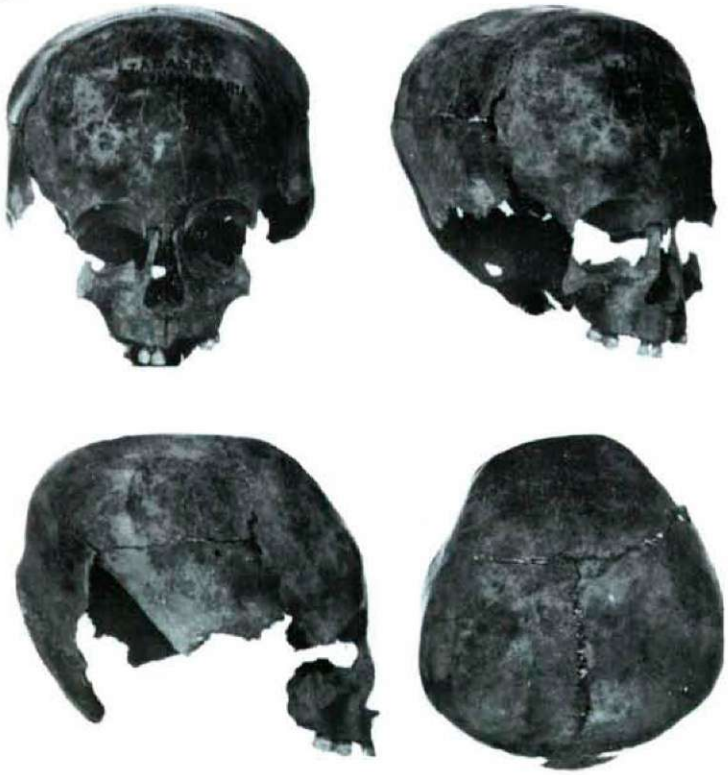

Fig. 2. Macrocephalic cranium of a child (Subotica—Hussarbarrack)

Above the tubera frontalia, at the bregma-region, the region above the lambda-point and that being in lateral direction from both of the branches of the sut. lambdoidea the cranium is extremely flat. That is why the tubera parietalia, tubera frontalia and the region in front of the bregma are considerable protruding. The way the deformation was completed is very similar to that of the cranium from Ada that will be desribed as follows.

Being of interest from the point of view of paleopathology, there are cribra orbitalia at both of the orbits, which may represent the cause of death.

Find-spot: Ada.

There are not any exact archeological data concerning this find. At the present time the cranium is deposited in the Woiwodina Museum (Novi Sad), its rescue is owing to the archeologist S. NAGY.

The calvaria in question is one with extremely strong walls, in very good state of preservation (Fig. 3). On the basis of the grade of obliteration of the sutura the age of death can be estimated at 75 years. The sexual coefficient is

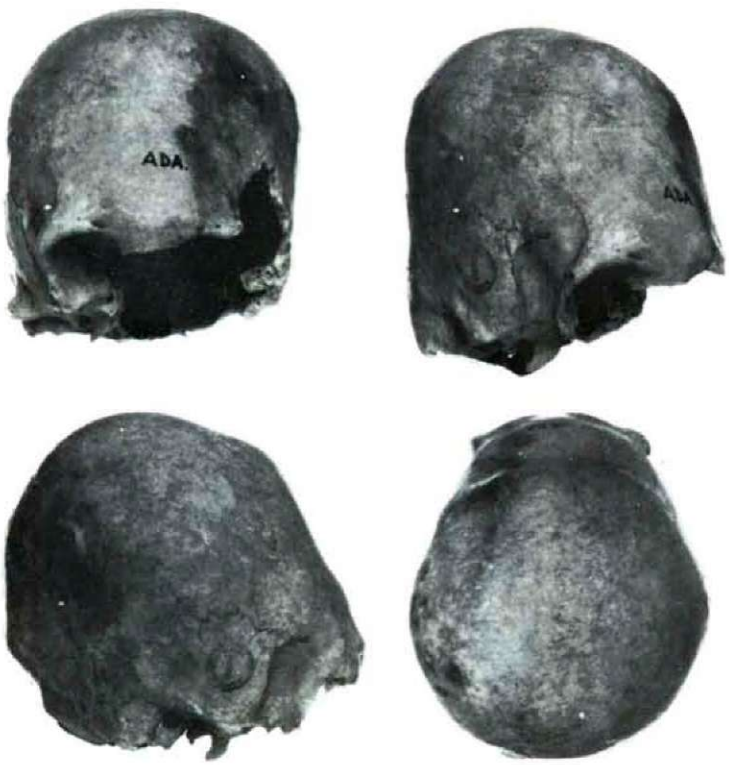

Fig. 3. Macrocephalic cranium of a male (Ada)

+ 1.5, that comes near to the complex of hypermasculine characteristics, so we determined it as a male.

On the basis of the absolute sizes, the cranium is short, medium wide, the forehead is narrow. According to the index it is hyperbrachycranic, stenometopic.

In the sut. lambdoidea there is ossa Wormiana. The occipital bone is cone-shaped.

Its deformation seems to be more considerable, than that in case of the find having come to light at Subotica-Sándor Co-operative Home. There is a fairly big hollow to be observed through the forehead and the bregma-region, as well as under the protuberantia occipitalis externa. Also in this case the deformation must have been a circular one, completed, however, by a different method. One of the bindings must have been through the frontal bone and under the protuberantia occipitalis externa. As at both the sut. lambdoidea and the protruding occipital bone there are flattening to be observed, we suppose that in this place the cranium was pressed down by three flat objects.

Find-spot: Bačka Topola.

The rescue of the finds (number 1 and 2) from an authentic Avar cemetery is due to the archeologist L. SZEKERES. Since 16th August 1958 they have been deposited in the Museum of Subotica.

Find No. 1 is a cranium in very good state of preservation (Fig. 4), its age of death can be estimated at 60 years, and its sex – according to the masculine and hypermasculine characteristics – can be determined as a male.

On the basis of absulote characteristics the cranium is of medium length, medium breadth, medium high, aristencephalic, the forehead is medium wide, bizygomatic breadth is large, the whole face and the upper-face are high, mesognathous.

On the basis of indices it is mesocranic, orthocranic, metriocranic, metriometopic, mesoprosopic, mesene, mesoconch, hyperchamaerrhine, brachystaphyline.

Fig. 4. Mongolid cranium of a male (Bačka Topola, No. 1)

In the area of palatum durum, at both sides of sut. palatina torus sagittalis of medium size has been found.

The alveolar part of maxilla is high and a well developed sulcus praenasalis can be felt. Great os epiptericum on the right pterion. Beneath the glabella supranasal remains of frontal bone. At the occlusion of the two rows of teeth there is psalidontia. The bones of the nose are relative wide and reach the area of os frontale.

As for taxonomy first of all Lipták's studies have been considered (LIPTÁK, 1959), according to which it bears the characteristics of the Sinid race belonging to the Mongolid race.

Find No. 2. is a cranium of very good preservation. On the basis of its characteristics the age at death is approximated as 29, the sex – calculated with the – 1,0 sexual ratio – is female.

Regarding the absolute measures of the crane it is of medium length widthand height, the forehead is narrow, bizigomatic breadth of medium width, the whole face and upper face hight, orthognathous.

From the indices it is brachycranic, orthocranic, tapeinocranic, stenometopic, mesoprosopic, leptene, hypsiconch, chamaerrhine, brachystaphyline.

On the right side of the sut. palatina mediana there is a weak torus palatinus sagittalis. The nasal bones are sand-clock-shaped and they stretch up to the frontal bone.

Taxonomically, the find mostly comes near to the Central Asian type of the Mongolids.

Significance of the finds

In our opinion, two from the three macrocephalic finds show the same deformation of lesser degree (Subotica-Hussarbarrack and Ada). In case of the third one (Sándor-Co-operative Home) a deformation of higher degree can be observed, completed by a different method. Accordingly, the finds can be divided into two groups.

There are a lot of macrocephalic find-spots we know in Hungary (PÁRDUCZ, 1963), especially near the big rivers (Danube, Tisza, Körös). Most of the find-spots having been discorvered near the Danube and the Tisza are determined as Hunnish, Gepid, furthermore, those near the Tisza as Avar or of any undetermined archeological period. Concerning our finds, that of Ada comes from a river region, whereas the finds of Subotica come from a farther district.

In Transdanubia, better to say near the Danube and along the Tisza two macrocephalic centres were formed in the migration period, differing from eachother in respect of the deforming instruments and methods (NEMESKÉRI, 1952). In case of the deformed crania of the Gepid cemetery of Kiszombor (BARTUCZ, 1936) the method of deformation comes near to that of the find from Ada. The cranium of a male we have described comes near to the material of Kiszombor, respectively the method as well as that the deformation used to be a habit chiefly in males (19 from 21 males). At the same time in case of the cranium of a Gepid female from Tápé the method deformation (FARKAS–LIPTÁK, 1971) comes near to that of the find of a female from Sándor Co-operative Home at Subotica.

From the point of view of physical anthropology the macrocephalic finds in Hungary were described by LIPTÁK in detail. He drawed attention to that most of the finds are of Europid character (LIPTÁK, 1961). At the same time the crania from Kiszombor show some Mongolid influence, that is why, in spite of their archeological material being a typical Germanic one, they are supposed to belong rather to a series of any Hunnish population (LIPTÁK, 1961). In case of the well preserved find of Subotica we are inclined to reveal, in addition to the predominance of Europid character, a very slight Mongolid influence, too.

Taking all these into account, we think that, though there are also some macrocephalic find-spots from the late Roman as well as the Hunnish periods in southern part of the area between the Danube and the Tisza, from the point of view of physical anthropology still our finds of undetermined archeological period can be connected chiefly with the Gepids. They are of importance first

Fig. 5. Mongolid cranium of a female (Bačka Topola, No. 2)

of all because they give some new datas referring to the macrocephalic find-spots in southern part of the area between the Danube and the Tisza.

The other two crania enrich the row of Avar series containing Mongolid components. Naturally, the proportion of the Mongolid elements in the cemetery cannot be determined at the present time. The cemeteries excavated between the Danube and the Tisza (LIPTÁK, 1959) – which include Mongolids, too –, as for instance the cemeteries of Madaras, Üllő I, Kiskőrös, appear to have been followed by some more find-spots in Jugoslavia (Čik, Bačka Topola).

At the moment the two Mongolid finds are important from the point of view of taking into account cemeteries like these. The complete anthropological evaluation of the Avar series semming to be of importance – which both of the crania belong to – is possible only by means of an excavation of a larger extent.

References

BARTUCZ, L. (1936) A kiszombori gepida temető koponyái (Die Gepida-Schädei des Gräberfeldes von Kiszombor). — Dolgozatok. *12*, 178—204.

FARKAS, GY.—LIPTÁK, P. (1971): A Tápé környéki leletek értékelése (Evalution of the finds from the outskirts of Tápé). In: JUHÁSZ, A. (edit.): Tápé története és néprajza (The history and the ethnography of Tápé). 163—167.

FARKAS, GY.—LENGYEL, I.—MARCSIK, A. (1972): Antropológiai praktikum I. Paleoantropológiai metodikák (Anthropological practice I. Paleoanthropological methods). 52—65.

LIPTÁK, P. (1959): The „Avar Period" Mongoloids in Hungary. — Acta Arch. Hung. 10. 251—279.

LIPTÁK, P. (1961): Germanische Skelettreste von Hács-Béndekpuszta aus dem 5. Jh. u. Z. — Acta Arch. Hung. *13*, 234—246.

LIPTÁK, P. (1971): Embertan és emberszármazástan (Anthropology and human evolution). — Bp. 234—236.

NEMESKÉRI, J. (1952): An anthropological examination of recent macrocephalic finds. — Acta Arch. Hung. *2*, 223—233.

PÁRDUCZ, M. (1963): Die ethnischen Probleme der Hunnezeit in Ungarn. — Studia Arch. 1. Bp.

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RECENT DATA ON THE ANTHROPOLOGY OF ONE OF THE POPULATIONS (VÉSZTŐ) OF THE HUNGARIAN GREAT PLAIN (ALFÖLD)

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Introduction

Under Turkish rule, the settlements of Békés county became partly or completely depopulated. Before the river Sebes Kőrös was controlled, the environment of Vésztő was characterized by the marshy landscape of the Kis-Sárrét; its resettling began in 1723, as a result of the activity of the Austrian János Harruckern. Harruckern followed the principle of settling a population of the same nationality and religion in each of the places earlier peopled. To Vésztő there went Hungarians of the reformed (Calvinist) religion, moving to their new residence mainly from Bihar and Hajdú counties in the eastern part of the Great Hungarian Plain (Alföld), and there also remigrated a large part of the population that had lived there earlier but made their escape from the Turkish occupation of the country. In this respect we refer partly to a geographical paper (PALOTÁS, A.: in the press.) and to a more detailed anthropological monograph (FARKAS—VARGA, in the press).

In addition to the investigation of the adult population, on the basis of the data of parish registers some notes were prepared by the authors as to the period between 1937—1970, as well. According to these, weddings took place mainly in the months of November and December, while in summer their number was very low. The frequency of births was highest in the month of September. 47,4 per cent of the deceased died due to diseases of the heart or circulatory disturbances. A wide range of cancerous diseases can be observed among the population. Most deaths occurred in March.

On the basis of the body measurements of 415 boys and 551 girls (body height, weight and normal chest measurement), the physical development of the 3—18 year old children at Vésztő can be described as good, even compared with the children in the large cities of Hungary. The menarche median of girls is 12.90 years, this does not fall behind the national value (BOTTYÁN *et al.*, 1963). The first menstrual bleeding occurred with the highest frequency in January.

Anthropological characterization of the adult population

The 903 adult individuals examined in March 1972 correspond to nearly 10% of the population. On the basis of the census data from 1970, the number of inhabitants was 9943 individuals (A. PALOTÁS, in the press). 77% of the persons measured, tracing their descent back to the grandparents, descended from families native to that place.

On the occasion of the anthropological investigations, the following measurements were determined: body height, trunk length, maximum head length, maximum head breadth, zygomatic breadth, morphological face height, angle breadth of the mandible, nasal height, nasal breadth (MARTIN–SALLER, 1957). The colours of hair and eyes too were established, on the basis of Saller's scale

of hair colours and Schultz's scale of eye colours. Finally, photographs of the persons were also made, in three norms (FARKAS–VARGA, in the press).

On the basis of the parameters calculated by the usual biometric methods the anthropological characterization of the adult population can be given as follows (Table 1 to 8).

Body height

The stature may be put, for both sexes, primarily in the lower categories. The low and small-medium stature occured for *males* (between 150–164 cm) in approximately 42%, and for *females* (between 140–153 cm) in about 47%. The frequency of those of large-medium and tall stature is for males (between 167–180 cm) approximately 35%, and for females (156–168 cm) about 32%.

The mean values of body height belong for males between 24–40 years of age to the large-medium group, and those between 41–60 years to the medium group. The average stature of the 24–40-year old females corresponds to the medium, and that of between 41–60 years of age to the small-medium body height.

The stature decreases with age. The difference in stature between the 16–23-year old individuals and those older than 61 years is 6.5 cm in the case of males, and 8.2 cm in the case of females.

Major measurements of the head

The maximum head length, for both sexes equally, is primarily medium, but the occurence of dolicho- and brachycephalic individuals is also considerable. On the basis of the maximum head breadth, in the case of both sexes, mainly the medium brachycephalic individuals are dominant, followed in frequency by the brachycephalic ones.

On the basis of the zygomatic breadth, the most frequent in the case of both sexes in the broad face, followed by the group of persons with medium broad faces. The ratio of broader faces is higher in females than in males. The morphological face height in both sexes, in approximately 90% of the examined persons, is established as low and medium high.

The most important indices

On the basis of the distribution of the *cephalic indices*, in both sexes, the brachy-, and hyperbrachycephalics are represented in 85–90%. The height-length index of the head shows a highly uniform picture in both sexes, nearly 100% of the examined persons belonging to the hypsicephalic group. According to the height-breadth index, there primarily metriocephaly occurs, but the number of acrocephalic individuals is considerable, too.

The values of the facial index indicate predominantly euryprosopy but a displacement can be observed in females mainly towards the lower, and in males towards the higher values. On the basis of the nasal index, the population is mainly leptorrhine, but mesorrhiny too is considerable.

Colour of eyes, colour of hair

The eyes of the males are generally of moderate pigmentation (light blue, light grey, greenish). Brown eyes could be observed in only 20% of the cases. In the case of the females, however, mainly shades of brown eye colours were found, in nearly 35%, and light blue and ash-blue eyes were extremely rare.

The hair, for both sexes, is mainly dark brown and black. Lighter brown hair too is considerable. Fair and red hair is very rare; it can be found in only a negligible proportion (in males 2.7%, and in females 5.4%).

On the basis of the two characteristics the joint occurence of black or dark brown hair and of greenish eyes is the most frequent for the males, and the combination of black or dark brown hair and greenish or brown eyes for females. The joint appearance of light hair and light blue eyes is in both sexes the most rarely.

Age differences

The difference of sature between males and females for the 24–60 year olds, but also for the older persons is almost uniformly 12 cm.

In males, after their 61st year of age, the length and contour of the head decrease. The mandibular angle breadth increases between 24 and 60 years as compared to the previous age-group, but after this it decreases and the mandible becomes narrower. The nasal breadth, like the nasal height, increases with the age. The facial height increases between 24–40 years, and then bocomes gradually lower. The same tendency can be observed in the facial index, too. That is to say, the face becomes lower not only in absolute measure but, at the same time, it becomes relatively broader, too.

The stature of the females, like that of the males, decreases with the age. The minimum frontal breadth does not change considerably in the individual age-groups. On the other hand, the angle breadth of the mandible increases more and more and the mandible becomes broader. The same refers to the nasal breadth, as well. The facial height increases somewhat between the years 24 to 40, and then begins to decrease.

As regards the other features, a considerable, constantly uniform difference cannot be demonstrated in any direction.

Measurement of variations

To establish the variability of the individual characteristics, we took Howell's „mean sigma" ratio as starting point. The formula underlying the calculation is: S. R. = 100. s/σ, which expresses the dispersion of the sample as a percentage of the dispersion considered as normal. The values obtained for the males are given in Table 9. The higher the value of S. R. above 100, the larger, the variation too, of course. It can be seen from the Table that the value of the ratio is lower than 100 only in the cases of the frontal and mandibular breadth, and for the other characteristics it is always higher. Even the average S. R. value for the seven absolute sizes exceeds 100. On this basis we may establish that for the given characteristics of the population investigated, a larger variation was found in the males than the average, indicating a moderately heterogeneous population.

Sexual dimorphism

The sexual dimorphism was extablished for absolute and relative dimensions on the basis of the sexual variance. It was determined with the previously applied formula (FARKAS–LIPTÁK, 1970). The sexual variance given in Table 10 for the absolute dimensions indicates that for the male and female individuals of the sample investigated a considerable sexual dimorphism was not found. The investigated population is in this respect fully similar to an earlier studied, similarly village population.

Taxonomic analysis

Taxonomic analysis is mainly of inportance in the investigation of paleoanthropological material (LIPTÁK, 1961). In an investigation of skeletal finds from the 7–13th centuries, the author cited gradually built up the important race-systematic places of human types and their somatologic characterization. These are as follows:

A B

Fig. 1

The characterization of the *Uralian* race, occurring mainly in the Ugrians, can be found in monographs on the Ostyaks (LIPTÁK, 1950), the Hungarian conquerors of the 10th century (LIPTÁK, 1954a), and the Ostyak population

Fig. 2

(*measured* by JÁNOS JANKÓ) that lived at the end of the last century (LIPTÁK, 1954b). The differentiation of the major *Europid races* was made in connection with the anthropological investigatin of the cemetery of the Hungarian common people from the 11th century, at Kérpuszta (LIPTÁK, 1953). The characterization of the *Turanids* and the verification of the existence of the Pamirian race among the anthropological components of the conquering Hungarians are contained in the same monograph, analysing mainly the taxonomic problems connected with the two races (LIPTÁK, 1955). The differential-diagnostic characterization of the Mongolid great race, and within this of the separate Mongolid taxons was published in connection with the Avars in Hungary (LIPTÁK, 1959). The anthroposystematics has been summarized on a number of occasions (LIPTÁK, 1962a; 1962 b; 1965; 1966; 1969).

On the basis of taxonomical standard works from 1955–1965, this could be applied, from the point of view of Hungarian ethnogenesis, to the paleoanthropological material.

The investigation of the populations living to-day from the taxonomic point of view is generally not such an important task. Nevertheless, it has some importance if, for example, we are looking for the continuity between – let us suppose – the present population and that in the Árpádian Age. This

A B

Fig. 3

historical continuity was often broken by historical processes of the inhabitants and demographical changes (transmigration, immigration, fluctuation in the number of the population, etc.). The territory of the Great Hungarian Plain is fundamentally important from the point of view of the settlement of the Hungarian population, but was exposed, unfortunately, to grave disasters like the invasion by the Tartars (1241–1242) and the Turkish rule for 150 years in Hungary after the defeat at Mohács (1526).

Another aim of taxonomic analysis is to enable the comparison of individual populations on the basis of some common principles – as a result of having separated them according to a certain point of view. The anthropological „type-spectra" of all peoples differ from one another, but even within one ethnic group some regional groups can be distinguished from each other. That is to say, the population of the Great Plain may show some differences, according to settlements of regions. Later on, we shall compare four settlements with that at Vésztő. It was mentioned in the Introduction that although the settlement at Vésztő belonged regionally to the area of the Great Plain at Nagysárrét, it developed from the adjacent settlements after the expulsion of the Turks. At the same time we can mention for comparison, that in Orosháza, for example, live Hungarians who migrated from the Transdanubian areas.

Let us now consider the anthropological make-up of the population investigated at Vésztő (Table 11).

A. The brachycephals of dark complexion are dominant (63%). Within these – and overall within the whole population – some 13% could not be diagnosed exactly (br). Among the brachycephalic anthropological components the Alpines (a) have the highest proportion. These are characterized by a rounded head and less strongly marked features, their stature being small-medium or medium. The profile of the nose is mostly concave. Good representatives of these are to be seen in Fig. 1 (pictures a and b). Very characteristic of this population is the Armenoid race (ar) that can be found in the conquering Hungarians, too. The nose is strongly protruding and curved, the tip of the nose tends somewhat downwards, and the front is sometimes curved; the so-called hawk nose is rather frequent. The stature is large-medium (Fig. 2). Very characteristic of the conquering Hungarians is the Pamirian race (p) originating from Middle Asia but with Europid features, such as the less protruding, somewhat curved nose and the flatness of the lambdoid region. In some cases there are added more or less Turanid features, as well (Fig. 3). In the population at Vésztő, particularly in the case of females, the fairly considerable proportion of the Lappid (l) race, called Protoalpine by some

A B

Fig. 4

anthropologist, was stirking. The Lappids are characterized by a low and and broad face, a definitely concave (snub) nose and a short stature. This feature is the most wide-spread among the Finno-Ugrians (Lapps) and it occurs in other regions of Eastern Europe, too, thus in Poland and the Ukraine (Fig. 4). Not numerous, but strongly marked representatives of the Dinaric (d) race could also be established. This human type is characterized by a large-medium or medium stature, flatness of the occiput (planoccipitalia) and a very strongly protruding, narrow, curved nose (Fig. 5).

Fig. 5

B. In this population the other human races appear in relatively not high proportions. Among them, however, the group of Cromagnoids with some 22% is outstanding; this name is due to their originating from the Upper-Palaeolithic Cro-Magnon race. Cromagnoid-A (crA) is the most reminiscent of this Upper Palaeolithic race. In the sample from Vésztő the participation of Cromagnoid-C (crC) is the most considerable (altogether some 10%), called also „Andronovo type" after a Bronze-Age site in Siberia. It is important to mention the „Andronovo race" – at least as a synonym – because this taxon is one of the components of the development of

Fig. 6

just the Turanid race of Europo-Mongolid character. In Hungary it presents itself generally in a non-depigmented form, its characteristic feature being a low, broad and oblong face (Fig. 6). In the earlier Hungarian anthropological

Fig. 7

literature there was no reference to the importance of this human type, this terminology not even being known at that time. Earlier a greater importance was attached to the type Cromagnoid-B (crB), or under a more well-known name, the East-Baltic or Eastern-Europid race, but its numerical proportion is not very considerable either in the present population or in that of the age of the Hungarian conquest. In the population of Vésztő, too, it means not more than some 8% (Fig. 7). It is characterized by a short or small-medium stature, a moderate brachycephaly, greyish eyes, pale fair hair and a concave noseprofile. It may have developed by hybridization. This race is otherwise characteristic of the Finns and of some Slavic peoples, too. Most reminiscent of the classical Cro-Magnon race is the Cromagnoid-A taxon. This can be proved, however, not more than about 4% of the population of Vésztő (iFg. 8). It differs from the Andronovo type mainly in that the head is longer and, according to the cephalic index, mesocephalic or, as a results of brachycephalisation, it is moderately brachycephalic.

Fig. 8

C. The Mediterranean race is also characteristic of the Hungarians, and it was characteristic in the age of the Hungarian conquest, too. This means altogether 13% of the population investigated at Vésztő. A somewhat higher numerical proportion than this points to the gracile Mediterranean (m) race (the male shown in picture „a" of Fig. 9), characterized by a dark complexion, and a small-medium or short stature. It is also characterized in a slightly lower proportion by the Atlanto-Mediterranean (am) taxon of taller stature (the female shown in picture „b" of Fig. 9).

A B

Fig. 9

D. Among the other elements of the population the very low numerical proportion of the dolichocephalic Nordics (n) of light complexion and tall stature is negligible. Much more important – having historic importance and indicative of the Hungarians of the age of conquest – are the individuals showing moderate Mongoloid features. In picture „a" of Fig. 10 the Ugrian origin of the Hungarians is represented by the female reminiscent of the Uralian race (u). In picture „b" of Fig. 10 we see a female similarly showing the features of the Europo-Mongolid Turanid (t) race and of the earlier-mentioned „andronovo type" of Europid character. This may have been called earlier the „race of the Great Plain" (Alföld type) by one of the prominent researchers of Hungarian ethnical anthropology, LAJOS BARTUCZ.

A B

Fig. 10

Comparison with other samples

We have compared the anthropological features of the population of Vésztő with other samples. In the comparison with various populations, a check was first made of the supposition of equal dispersions by the F-test. The 2% level was used, and where the hypothesis of equal dispersions had to be rejected at that level, the t-test was not performed. The calculated values are given on the basis of variance analysis in Tables 12 and 13, and on that of arithmetic means in Tables 14 and 15. For comparison we have used the samples of Tápé (FARKAS–LIPTÁK, 1970), Orosháza (FARKAS–LIPTÁK, 1965), Dömsöd (KELEMEN, 1968) and Szakmár (HENKEY, 1966). On the basis of our calculations the following can be established:

a. There is a major difference between the averages that can be proved statistically, too (at a 99% confidence level, with the value $t = 2{,}58$, $P < 1\%$) in the following cases.

Males: as compared with the males of Vésztő, the following measurements are larger: the maximum head breadth, the minimum frontal breadth, the zygomatic breadth, the morphological face height and the cephalic index of the males of Szakmár; the maximum head length and the zygomatic breadth of the males of Tápé; the stature of those of Orosháza; the minimum frontal

breadth and the transverse fronto-parietal index of those of Dömsöd; on the other hand, the cephalic indices of the males of Tápé and Orosháza are smaller than those of the males of Vésztő.

Females: as compared with females of Vésztő, the following measurements are larger: the angle breadth of the mandible, the face height and the stature of those of Orosháza; the minimum frontal breadth and the transverse fronto-parietal index of the females of Dömsöd; on the other hand, the angle-breadth of the mandible of the females at Vésztő is larger the than that of the females at Orosháza.

b. There is a statistically provable difference between the averages (at a 95% confidence level, with the value $t = 1.96$, $1\% < P < 5\%$) in the following cases.

Females: the maximum head length of the females of Szakmár is larger and the cephalic index of those of Tápé is smaller, as compared to the females of Vésztő.

c. In a great number of the cases the t-test could not be applied (these t-values are missing from the corresponding columns of Tables 14 and 15).

d. Finally, in the other cases no statistically provable difference ($P > 5\%$) can be demonstrated between the arithmetic means.

On the basis of all this, it seems that there is no difference from an anthropological point of view between the males and females of Vésztő and Dömsöd. The samples of Vésztő and Tápé are very close to each other, while in the case of the males and females of Vésztő and Orosháza, as well as in that of the females of Szakmár, owing to the different character of dispersions, the difference between the means cannot be evaluated statistically. This does not mean that these samples should be identical as the difference between the means cannot be proved. The problem is only that the possibly existing difference cannot be demonstrated on the basis of the arithmetic means. If there is really any difference between the samples mentioned in these cases, too, then it can be established only on the basis of the dispersions. For these investigations we would need the original data, and in addition, the problem could be solved only by performing Mann-Whitney's test (Hajtman, 1968), taking into consideration the large number of the elements of the samples. We can mention as an example the empirical dispersion curve of the cephalic index

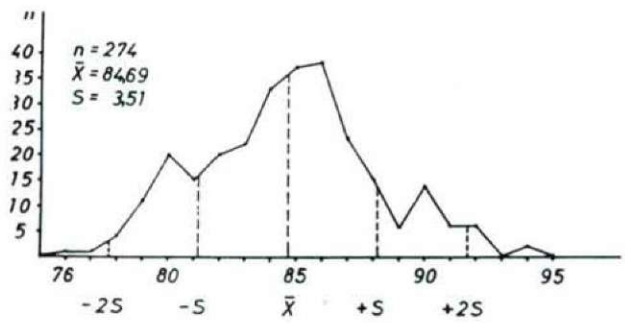

Fig. 11. Empirical distribution of the cephalic indices of 24—60-years old males

that, at least for males, is indicative of the presence of three components (Fig. 11), while for females (Fig. 12) it strongly approaches the Gaussian curve for normal dispersion. It is conceivalbe, therefore, that the very similar arithmetic means of the various samples refer to groups of entirely different composition.

We have carried out the comparison of populations on a taxonomic basis, as well. It meant some difficulty that, except for the samples of Orosháza,

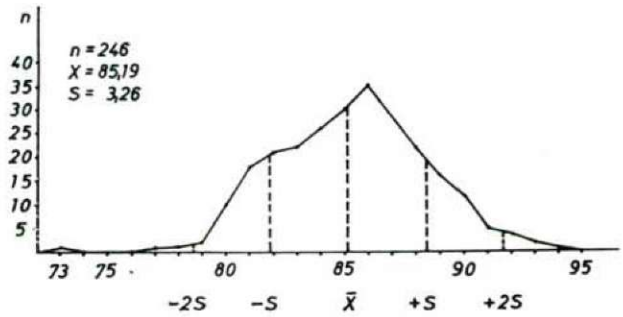

Fig. 12. Empirical distribution of the cephalic indices of 24—60-years old females

Tápé and Vésztő, detailed taxonomic tables had not been published; attention was drawn only to the most important components or the percentage of these were given. Our taxonomic comparison was therefore limited mainly to the three samples mentioned.

The result of this comparison is as follows: in the population of Orosháza (number of sample elements: 683) the brachycephalic individuals prevail (64%), after these, the Mediterranean ones have also a major share (23%). In the sample of Tápé (number of the sample elements: 686) the brachycephals similarly dominate, their percentage, however, being lower (45%), while the next most important component is not the Mediterranean but the Cromagnoid (34%). In the population of Vésztő the occurence of brachycephals is of approximately the same percentage as in the population of Orosháza, and the Cromagnoids appear with an even greater frequency (Table 11).

In the case of the three samples from the southern region of the Great Plain (total number of sample elements: 2107) the brachycephalic group occured in the most considerable proportion (58%), followed by the Cromagnoid (22%) and Mediterranean groups (17%). The frekuencies of the Nordic race (4%) and of the Mongoloid elements (1%) were negligible. Particularly the latter is remarkable, as according to GYULA HENKEY's investigations on the Danube-Tisza interstream region the occurence of the Europo-Mongolid (primarily Turanid) elements in some 40% is characteristic of the Hungarians. It might be admitted that in the area investigated by him (the Danube–Tisza interstream region) the frequency of these is higher than in other territories of the Great Plain. All the same, such a large difference must mainly be the result of taxonomic analysis by another method – as referred to by us already in an earlier paper (FARKAS–LIPTÁK, 1970).

References

BOTTYÁN, O.—DEZSŐ, GY.—EIBEN, O.—FARKAS, GY.—RAJKAI, T.—THOMA, Á.—VÉLI, GY. (1963): A menarche kora Magyarországon. (The age of menarche in Hungary). — Anthrop. Közl. *7*, 25—39.

FARKAS, Gy.—LIPTÁK, P. (1965): A lakosság embertani képe. In: Orosháza története és néprajza. (Anthropological picture of the population. A history and ethnography of Orosháza). Red.: Gyula Nagy. Orosháza. *2*. 344—399.

FARKAS, Gy.—LIPTÁK, P. (1970): Újabb adatok a magyarság etnikai embertanához. Tápé népességének anthropológiai vizsgálata. (Neuere Angaben zur ethnischen Anthropologie des Ungartums. Anthropologische Untersuchung der Population von Tápé). Anthrop. Közl. *14*, 35—70.

FARKAS, Gy.—VARGA, I. (in the press): Vésztő lakosságának antropológiai arculata. Vésztő nagyközség monográfiája. (Anthropological aspect of the population of Vésztő. — In: A monograph of the community Vésztő).

HAJTMAN, B. (1966): Bevezetés a matematikai statisztikába pszichológusok számára. (Introduction to mathematical statistics for psychologists). — Budapest. 348—352.

HENKEY, Gy. (1966): Szakmár felnőtt lakosságának embertani vizsgálata. (Anthropologische Untersuchung der Erwachsenen von Szakmár). In: „Szakmári honismereti értesítő". 35—55.

KELEMEN, A. (1968): Dömsöd, egy központi fekvésű község népességének embertani helye. (Dömsöd, position anthropologique d'une population centrale de la Hongrie). — Anthrop. Közl. *12*, 125—160.

LIPTÁK, P. (1950): Materialy po kraniologii Hantov (Étude anthropologique des crânes ostiaks). — Acta Ethn. Hung. *1*, 197—230.

LIPTÁK, P. (1953): L'analyse typologique de la population de Kérpuszta au Moyen Age. — Acta Arch. Hung. *3*, 303—370.

LIPTÁK, P. (1954a): An Anthropological Survey of Magyar Prehistory. — Acta Linguist. Hung. *4*, 133—170.

LIPTÁK, P. (1954b): JANKÓ JÁNOS vizsgálatai a Közép-Ob melléki chantik között. (J. JANKO's investigations among the Hantis along the Middle-Ob). — Nyelvtud. Közl. *56*, 97—116.

LIPTÁK, P. (1955): Zur Frage der anthropologischen Beziehungen zwischen dem Mittleren Donaubecken und Mittel-asien. — Acta Orient. Hung. *5*, 271—312.

LIPTÁK, P. (1959): The „Avar Period" Mongoloids in Hungary. — Acta Arch. Hung. *10*, 250—279.

LIPTÁK, P. (1961): On the problems of historical anthropology (palaeoanthropology). — Acta Biol. Szeged. *7*, 175—183.

LIPTÁK, P. (1962a): Embertan és emberszármazástan. (Anthropology and human origins). — Univ. lecture notes. Budapest.

LIPTÁK, P. (1962b): Homo sapiens — species collectiva. — Anthrop. Közl. *6*, 17—27.

LIPTÁK, P. (1965): On the taxonomic method in palaeoanthropology (historical anthropology). — Acta Biol. Szeged. *11*, 169—183.

LIPTÁK, P. (1966): Embertan és emberszármazástan. (Anthropology and human origins). — Uniform Univ. lecture notes. Budapest.

LIPTÁK, P. (1969): Embertan és emberszármazástan. (Anthropology and human origins). — — Univ. Textbook. Budapest. (2nd ed. 1971).

MARTIN, R.—SALLER, K. (1957): Lehrbuch der Anthropology. Stuttgart.

A. PALOTÁS, J. (in the press): Vésztő nagyközség gazdaságföldrajzi viszonyai. — In: Vésztő nagyközség monográfiája. (Economic geographic conditions of the community of Vésztő. — In: A monograph of the community of Vésztő).

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Table 1. Major parameters of the measurements and indices of the 24—40-years old males

No.of measu-rement	Character	n	w	$\bar{x}$	m	s^2	s
1.	Maximum head length	90	176—202	186,95	0,62	34,32	5,85
3.	Maximum head breadth	90	146—176	158,57	0,58	31,02	5,56
4.	Minimum frontal breadth	90	100—121	108,31	0,44	17,54	4,18
6.	Bizygomatic breadth	91	133—162	142,72	0,49	22,23	4,71
8.	Mandibular angle breadth	91	102—128	111,00	0,55	27,66	5,25
13.	Nasal breadth	91	30— 41	35,25	0,24	5,44	2,33
15.	Head-ear height	91	119—145	131,72	0,60	33,10	5,75
18.	Morphological face height	91	106—140	121,33	0,74	50,06	7,07
21.	Nasal height	91	44— 62	52,91	0,36	12,16	3,48
45.	Horizontal head contour	91	500—595	562,61	1,71	67,34	16,35
1.	Body height	88	154,9—184,5	167,93	0,62	34,04	5,83
23.	Sitting height (trunk length)	91	81,0— 97,3	87,51	0,34	10,77	3,28
3:1	Cephalic index	89	77,7— 92,2	84,77	0,35	10,71	3,27
15:1	Heigh-length index	90	62,3— 80,7	70,70	0,38	10,71	3,64
15:3	Heigh-breadth index	90	75,5— 93,9	83,38	0,41	13,22	3,86
4:3	Transverse frontoparietal index	89	62,5— 75,8	68,59	0,31	8,59	2,93
18:6	Morphological facial index	91	72,9—102,1	85,16	0,58	30,74	5,54
8:6	Jugomandibular index	91	66,1— 87,4	77,73	0,36	11,58	3,40
13:21	Nasal index	91	50,0— 82,0	66,83	0,67	41,15	6,41

Table 2. Major parameters of the measurements and indices of the 24—40-year old females

No. of measu-rement	Character	n	w	$\bar{x}$	m	s^2	s
1.	Maximum head length	96	164—194	178,20	0,55	30,10	5,48
3.	Maximum head breadth	96	137—169	151,50	0,59	34,01	5,83
4.	Minimum frontal breadth	96	95—118	104,57	0,49	22,95	4,79
6.	Bizygomatic breadth	96	123—154	136,52	0,56	30,67	5,53
8.	Mandibular angle breadth	96	92—120	103,12	0,57	31,05	5,57
13.	Nasal breadth	96	26— 40	32,41	0,25	6,07	2,46
15.	Head-ear height	96	117—145	127,47	0,57	30,88	5,55
18.	Morphological face height	96	96—123	110,34	0,61	35,77	5,98
21.	Nasal height	96	42— 60	49,66	0,33	10,50	3,24
45.	Horizontal head contour	96	515—590	542,62	1,51	218,97	14,79
1.	Body height	95	141,7—168,7	155,61	0,56	30,33	5,50
23.	Sitting height (trunk length)	95	72,2— 91,2	82,71	0,34	11,35	3,36
3:1	Cephalic index	96	72,5— 93,4	85,03	0,34	11,48	3,38
15:1	Height-length index	96	65,1— 77,7	71,56	0,31	9,08	3,01
15:3	Height-breadth index	96	76,9— 92,2	84,09	0,26	13,38	3,51
4:3	Transverse frontoparietal index	96	61,2— 77,6	69,10	0,34	11,29	3,36
18:6	Morphological facial index	96	69,1— 93,7	80,86	0,51	24,91	4,99
8:6	Jugomandibular index	96	68,3— 83,5	76,10	0,33	10,79	3,28
13:21	Nasal index	96	49,1— 86,4	65,62	0,69	45,61	6,75

Table 3. Major parameters of the measurements and indices of the 41—60-year old males

No. of measurement	Jelleg	n	w	$\bar{x}$	m	s^2	s
1.	Maximum head length	185	170—201	186,10	0,48	42,69	6,53
3.	Maximum head breadth	185	141—172	157,44	0,40	29,79	5,45
4.	Minimum frontal breadth	185	89—122	108,02	0,37	26,04	5,10
6.	Bizygomatic breadth	185	129—159	142,95	0,40	29,49	5,43
8.	Mandibular angle breadth	185	94—129	111,95	0,41	31,47	5,60
13.	Nasal breadth	185	30— 47	36,56	0,23	9,67	3,11
15.	Head-ear height	185	111—149	129,63	0,45	37,57	6,14
18.	Morphological face height	185	102—138	120,13	0,48	42,76	6,53
21.	Nasal height	185	47— 62	53,51	0,23	10,23	3,19
45.	Horizontal head contour	185	519—600	558,57	1,13	237,14	15,39
1.	Body height	180	146,2—177,7	164,93	0,44	34,74	5,89
23.	Sitting height (trunk length)	184	75,5— 93,5	86,04	0,23	9,83	3,13
3:1	Cephalic index	185	76,4— 94,4	84,66	0,26	13,18	3,63
15:1	Height-length index	185	61,0— 78,7	70,13	0,23	10,05	3,16
15:3	Height-breadth index	185	73,9— 94,3	82,89	0,28	14,60	3,82
4:3	Transverse frontoparietal index	185	57,8— 75,9	68,71	0,24	10,34	3,21
18:6	Morphological facial index	185	71,0—100,8	84,14	0,37	26,14	5,11
8:6	Jugomandibular index	185	67,8— 87,7	78,29	0,24	11,13	3,33
13:21	Nasal index	185	55,6— 87,2	68,56	0,52	51,16	7,15

Table 4. Major parameters of the measurements and indices of the 41—40-year old females

No. of measurement	Character	n	w	$\bar{x}$	m	s^2	s
1.	Maximum head length	150	160—194	177,47	0,46	31,93	5,65
3.	Maximum head breadth	150	137—165	151,29	0,43	28,59	5,34
4.	Minimum frontal breadth	150	94—115	104,66	0,33	16,57	4,07
6.	Bizygomatic breadth	150	124—152	136,26	0,41	25,52	5,05
8.	Mandibular angle breadth	150	92—119	104,15	0,40	24,39	4,93
13.	Nasal breadth	150	27— 41	33,18	0,20	6,57	2,56
15.	Head-ear height	149	105—141	126,23	0,45	31,25	5,59
18.	Morphological face height	140	90—126	109,81	0,48	35,11	5,92
21.	Nasal height	150	40— 57	48,95	0,26	9,73	3,11
45.	Horizontal head contour	150	500—578	542,47	1,17	206,43	14,36
1.	Body height	147	140,9—165,7	152,56	0,43	27,22	5,21
23.	Sitting height (trunk length)	146	71,1— 90,0	81,67	0,28	11,57	3,40
3:1	Cephalic index	150	76,8— 93,9	85,30	0,26	10,12	3,18
15:1	Height-length index	149	63,4— 79,3	71,31	0,25	9,39	3,06
15:3	Height-breadth index	149	65,2— 92,9	83,48	0,32	15,35	3,91
4:3	Transverse frontoparietal index	150	63,1— 76,6	69,22	0,22	7,59	2,75
18:6	Morphological facial index	150	67,9— 95,4	80,78	0,39	22,52	4,74
8:6	Jugomandibular index	150	68,1— 83,6	76,37	0,26	10,17	3,18
13:21	Nasal index	150	54,9— 83,7	68,03	0,50	37,79	6,14

Table 5. Major parameters of the measurements and indices of the males over 61

No. of measurement	Character	n	w	$\bar{x}$	m	s^2	s
1.	Maximum head length	189	158—204	184,07	0,47	41,83	6,46
3.	Maximum head breadth	189	139—174	156,57	0,45	37,81	6,14
4.	Minimum frontal breadth	189	96—118	106,06	0,35	22,91	4,78
6.	Bizygomatic breadth	189	127—167	141,85	0,43	34,64	5,98
8.	Mandibular angle breadth	189	99—129	111,41	0,45	37,84	6,15
13.	Nasal breadth	189	31— 50	37,43	0,26	12,53	3,53
15.	Head-ear height	189	114—143	129,88	0,41	32,70	5,71
18.	Morphological face height	189	103—138	118,96	0,54	54,47	7,38
21.	Nasal height	189	47— 69	54,51	0,27	13,50	3,67
45.	Horizontal head contour	189	518—605	553,84	1,14	247,93	16,71
1.	Body height	172	146,7—176,7	161,47	0,48	40,39	6,37
23.	Sitting height (trunk length)	181	72,2— 93,2	83,52	0,27	13,19	3,63
3:1	Cephalic index	189	74,2— 97,5	85,13	0,26	14,23	3,77
15:1	Height-length index	189	63,4— 82,0	70,62	0,23	10,04	3,16
15:3	Height-breadth index	189	75,0— 97,2	83,05	0,26	12,74	3,56
4:3	Transverse frontoparietal index	189	60,0— 77,1	67,82	0,20	7,51	2,74
18:6	Morphological facial index	189	71,4— 95,5	83,63	0,38	27,35	5,22
8:6	Jugomandibular index	189	70,2— 89,0	78,57	0,27	10,03	3,74
13:21	Nasal index	189	54,7— 92,5	68,91	0,53	53,17	7,29

Table 6. Major parameters of the measurements and indices of the females over 61

No. of measurement	Character	n	w	$\bar{x}$	m	s^2	s
1.	Maximum head length	74	161—190	178,35	0,64	30,21	5,49
3.	Maximum head breadth	74	141—165	151,28	0,52	20,35	4,51
4.	Minimum frontal breadth	74	93—112	104,26	0,50	18,96	4,35
6.	Bizygomatic breadth	74	126—145	135,81	0,55	22,35	4,72
8.	Mandibular angle breadth	74	92—120	104,69	0,66	32,11	5,66
13.	Nasal breadth	74	27— 42	34,42	0,32	7,73	2,77
15.	Head-ear height	74	116—145	127,92	0,69	35,42	5,95
18.	Morphological face height	74	91—123	108,09	0,82	50,42	7,10
21.	Nasal height	74	44— 58	50,35	0,33	6,34	2,88
45.	Horizontal head contour	74	520—589	543,44	1,64	200,15	14,14
1.	Body height	72	13,57—159,5	149,14	0,63	29,05	5,38
23.	Sitting height (trunk length)	73	70,7— 85,7	78,81	0,45	14,66	3,82
3:1	Cephalic index	74	77,0— 93,3	84,92	0,36	9,80	3,13
15:1	Height-length index	74	64,2— 78,9	71,78	0,39	11,33	3,36
15:3	Height-breadth index	74	76,6— 95,4	84,68	0,45	15,46	3,92
4:3	Transverse frontoparietal index	74	62,8— 74,8	68,96	0,32	7,57	2,75
18:6	Morphological facial index	74	67,6— 91,6	79,94	0,62	28,36	5,32
8:6	Jugomandibular index	74	68,4— 86,3	77,05	0,41	12,63	3,55
13:21	Nasal index	74	56,0— 91,3	68,72	0,71	37,59	6,13

Table 7. Frequency of the major index groups. — Males

Index	Graduation	Denomination	24—60 years		Over 61		Total	
			n	%	n	%	n	%
Cephalic index	x—75,9	Dolichocephalic	—	—	1	0,5	1	0,2
	76,0—80,9	Mesocephalic	40	14,5	25	13,3	73	14,2
	81,0—85,4	Brachycephalic	121	44,0	73	38,8	221	42,9
	85,5—x	Hyperbrachycephalic	114	41,4	89	47,3	220	42,7
		Total:	275		188		515	
Height-length index	57,7—62,5	Orthocephalic	2	0,7	—	—	2	0,4
	62,6—x	Hypsicephalic	274	99,3	188	100,0	514	99,6
		Total:	276		188		516	
Height-breadth index	x—78,9	Tapeinocephalic	41	14,8	23	12,2	69	13,4
	79,0—84,9	Metriocephalic	153	55,4	112	59,6	296	57,4
	85,0—x	Acrocephalic	82	29,7	53	28,2	151	29,2
		Total:	276		188		516	
Morphological facial index	x—78,9	Hypereuryprosopic	37	13,4	29	15,4	78	15,1
	79,0—83,9	Euryprosopic	98	35,4	72	38,3	188	36,4
	84,0—87,9	Mesoprocopic	73	26,3	40	21,3	128	24,7
	88,0—92,9	Leptoprosopic	55	19,8	39	20,7	98	18,9
	93,0—x	Hyperleptoprosopic	14	5,0	8	4,3	25	4,8
		Total:	277		188		517	
Nasal index	x—54,9	Hyperleptorrhine	2	0,7	2	1,1	5	1,0
	55,0—69,9	Leptorrhine	169	61,0	106	56,4	307	59,4
	70,0—84,9	Mesorrhine	103	37,2	74	39,3	196	37,9
	85,0—99,9	Chamaerrhine	3	1,1	6	3,2	9	1,7
		Total:	27		188		517	

Table 8. Frequency of the major index groups. — Females

Index	Graduation	Denomination	24—60 years		Over 61		Total	
			n	%	n	%	n	%
Cephalic index	x—75,9	Dolichocephalic	1	0,4	—	—	1	0,3
	76,0—80,9	Mesocephalic	20	8,1	6	8,1	33	8,5
	81,0—85,4	Brachycephalic	110	44,7	35	47,3	175	45,3
	85,5—x	Hyperbrachycephalic	116	46,7	33	44,6	177	45,9
		Total:	246		74		386	
Height-length	62,6—x	Hypsicephalic	245	100,0	74	100,0	385	100,0
		Total:	245		74		385	
Height-breadth index	x—78,9	Tapeinocephalic	22	9,0	5	6,8	33	8,6
	79,0—84,9	Metriocephalic	136	55,5	34	45,9	213	55,3
	85,0—x	Acrocephalic	87	35,5	35	47,3	139	36,1
		Total:	245		74		385	
Morphological facial index	x—78,9	Hypereuryprosopic	86	34,9	32	43,2	141	36,5
	79,0—83,9	Euryprosopic	100	40,6	24	32,4	152	39,4
	84,0—87,9	Mesoprosopic	44	17,9	13	17,6	70	18,1
	88,0—92,9	Leptoprosopic	11	4,5	5	6,8	18	4,7
	93,0—x	Hyperleptoprosopic	5	2,0	—	—	5	1,3
		Total:	246		74		386	
Nasal index	x—54,9	Hyperleptorrhine	6	2,4	—	—	8	2,1
	55,0—69,9	Leptorrhine	158	64,2	45	60,8	251	65,0
	70,0—84,9	Mesorrhine	81	32,9	28	37,8	125	32,4
	85,0—99,9	Chamaerrhine	1	0,4	1	1,4	2	0,5
		Total:	246		74		386	

Table 9. Values of the sigma ratio of 24—60-years old males at Vésztő

No. of measurement	Character	S. R.
1.	Maximum head length	102,10
3.	Maximum head breadth	105,76
4.	Minimum frontal breadth	98,37
6.	Bizygomatic breadth	102,45
8.	Mandibular angle breadth	95,00
18.	Morphological face height	105,31
1.	Body height	104,13
		S.R. = 101,87
3:1	Cephalic index	103,23
18:6	Morphological facial index	103,52
		S.R. = 103,37

Table 10. Parameters of sexual dimorphism in the sample of Vésztő

No. of measurement	Character	d^2
1.	Maximum head length	5,62
3.	Maximum head breadth	4,33
4.	Minimum frontal breadth	2,69
6.	Bizygomatic breadth	5,20
8.	Mandibular angle breadth	13,39
13.	Nasal breadth	22,18
15.	Head-ear height	2,56
18.	Morphological face height	20,70
21.	Nasal height	15,92
45.	Horizontal head contour	2,50
1.	Body height	14,44
23.	Sitting height	6,97
		V_{sex} = 9,71
3:1	Cephalic index	0,08
15:1	Height-length index	0,59
15:3	Height-breadth index	0,34
4:3	Transverse frontoparietal index	0,13
18:6	Morphological facial index	4,93
8:6	Jugomandibular index	1,42
13:21	Nasal index	0,45
		V_{sex} = 1,13

Table 11. Result of taxonomic analysis in the sample of Vésztő

Races, sub-races		Males		Females		Totel	
		n	%	n	%	n	%
Brachycephals of dark complexion	Alpian (a)	69	15,5	71	24,1	140	18,9
	Armenoid (ar)	84	18,9	39	13,3	123	16,7
	Pamirian (p)	50	11,3	12	4,1	62	8,4
	Lappid (l)	5	1,1	30	10,2	35	4,7
	Dinaric (d)	11	2,5	4	1,4	15	2,0
	Undetermined brachycephalic (br)	63	14,2	29	9,9	92	12,5
	Total:	282	63,5	185	62,9	467	63,3
Cromagnoids	Cromagnoid—C (crC)	38	8,6	33	11,2	71	9,6
	Cromagnoid—B (crB)	28	6,3	29	9,9	57	7,7
	Cromagnoid—A (crA)	17	3,8	14	4,8	31	4,2
	Total:	83	18,7	76	25,8	159	21,5
Mediterraneans	Gracile Mediterranean (m)	46	10,4	11	3,7	57	7,7
	Atlanto-Mediterranean (am)	24	5,4	14	4,8	38	5,1
	Total:	70	15,8	25	8'5	95	12,9
Nordics (n)		8	1,8	3	1,0	11	1,5
Europids with Mongoloid features		1	0,2	5	1,7	6	0,8
	Total:	444		294		738	

Table 12. Major parameters of the 24—60-years old males in five different

No. of measurement	Character	Vésztő			Tápé			Orosháza			Dömsöd			Szakmár		
		n	$\bar{x}$	s^2	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2
1.	Maximum head length	275	186,38	40,11	242	188,09	38,06	1017	187,17	42,97	221	186,15	39,89	231	185,58	34,60
3.	Maximum head breadth	275	157,81	30,30	242	157,61	28,09	1015	156,15	40,08	220	157,29	35,30	231	160,99	27,56
4.	Minimum frontal breadth	275	108,11	23,24	242	108,50	21,81	1012	109,35	29,04	219	110,59	23,26	231	112,02	20,18
6.	Bizygomatic breadth	276	142,72	27,39	242	144,25	33,18	1012	141,41	62,25	221	143,30	30,37	230	145,70	29,26
8.	Mandibular angle breadth	276	111,64	30,41	242	111,54	43,03	1019	110,46	44,73	221	112,98	44,88	231	112,08	33,17
18.	Morphological face height	276	120,52	45,48	242	120,19	46,38	1016	120,25	50,20	220	120,78	55,11	231	123,02	43,00
1.	Body height	268	165,91	36,49	241	166,85	38,56	1017	167,14	41,98	218	166,51	42,79	231	168,87	34,37
3:1	Cephalic index	274	84,69	12,37	242	83,91	11,02	1015	83,57	14,71	221	84,58	12,42	231	86,76	13,16
4:3	Transverse frontoparietal index	274	68,67	9,77	242	68,83	6,95	1011	70,07	14,57	219	70,41	10,39	—	—	—
18:6	Morphological facial index	276	84,48	27,89	242	83,47	26,83	1009	85,29	37,89	220	84,16	28,25	230	84,46	24,47

Table 13. Major parameters of the 24—60 years olf females in five different

No. of measurement	Character	Vésztő			Tápé			Orosháza			Dömsöd			Szakmár		
		n	$\bar{x}$	s^2	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2
1.	Maximum head length	246	177,75	31,34	270	179,22	30,10	336	178,49	39,60	131	178,27	36,70	231	178,89	27,55
3.	Maximum head breadth	246	151,37	30,72	270	151,68	25,98	334	150,97	33,64	131	152,06	32,53	232	155,28	17,36
4.	Minimum frontal breadth	246	104,62	19,06	270	104,73	15,48	337	106,16	31,27	130	107,52	24,38	232	109,53	14,29
6.	Bizygomatic breadth	246	136,36	27,54	270	135,87	23,35	336	131,90	73,71	131	135,90	30,34	232	139,39	21,63
8.	Mandibular angle breadth	246	103,75	27,24	270	103,78	26,49	338	102,31	33,36	131	104,05	25,68	232	105,16	23,09
18.	Morphological face height	246	110,02	35,43	269	110,06	30,28	336	109,18	43,19	131	111,16	63,03	232	113,55	29,30
1.	Body height	242	153,76	30,66	269	154,35	33,41	336	155,98	33,18	130	154,30	41,79	232	157,65	25,44
3:1	Cephalic index	246	85,19	10,66	270	84,59	8,95	336	84,64	11,49	131	85,18	11,68	231	86,85	7,83
4:3	Transverse frontoparietal index	246	69,17	9,04	270	69,09	7,18	333	70,23	14,99	130	70,75	9,32	—	—	—
18:6	Morphological facial index	246	80,81	23,18	269	81,05	18,57	334	83,09	39,43	131	81,89	33,44	232	81,55	17,69

Table 14. Results of the F- and t-tests in the case of the four materials investigated. — Males

No. of measurement	Character	Vésztő—Szakmár		Vésztő—Tápé		Vésztő—Orosháza		Vésztő—Dömsöd	
		F	t	F	t	F	t	F	t
1.	Maximum head length	1,15	1,48	1,05	3,17	1,07	1,84	1,00	0,40
3.	Maximum head breadth	1,10	6,76	1,08	0,42	1,32	—	1,16	1,02
4.	Minimum frontal breadth	1,15	9,53	1,06	0,93	1,24	—	1,13	5,63
6.	Bizygomatic breadth	1,07	6,47	1,21	3,25	2,27	—	1,11	1,23
8.	Mandibular angle breadth	1,09	0,88	1,41	—	1,47	—	1,47	—
18.	Morphological face height	1,05	4,23	1,01	0,55	1,10	0,57	1,21	0,41
1.	Body height	1,06	5,58	1,05	1,74	1,15	2,86	1,17	1,05
3:1	Cephalic index	1,06	6,90	1,12	2,60	1,19	4,66	1,00	0,37
4:3	Transverse frontoparietal index	—	—	1,40	—	1,49	—	1,06	6,21
18:6	Morphological facial index	1,14	0,04	1,04	2,24	1,36	—	1,01	0,68

Table 15. Results of the F- and t-tests in the case of the four materials investigated. — Females

No. of measurement	Character	Vésztő—Szakmár		Vésztő—Tápé		Vésztő—Orosháza		Vésztő—Dömsöd	
		F	t	F	t	F	t	F	t
1.	Maximum head length	1,13	2,28	1,04	3,06	1,26	1,48	1,17	0,84
3.	Maximum head breadth	1,77	—	1,18	0,67	1,09	0,87	1,06	1,15
4.	Minimum frontal breadth	1,33	—	1,23	0,30	1,64	—	1,27	5,80
6.	Bizygomatic breadth	1,27	—	1,17	1,11	2,67	—	1,10	0,81
8.	Mandibular angle breadth	1,18	3,13	1,03	0,07	1,22	3,13	1,06	0,54
18.	Morphological face height	1,21	6,92	1,17	0,80	1,21	1,61	1,77	—
1.	Body height	1,20	8,10	1,09	1,18	1,08	4,72	1,36	—
3:1	Cephalic index	1,36	—	1,19	2,14	1,08	1,96	1,09	0,02
4:3	Transverse frontoparietal index	—	—	1,25	—	1,66	—	1,03	4,78
18:6	Morphological facial index	1,31	—	1,24	0,40	1,70	—	1,44	—

Anthropological features og the persons in thfl photograph Plates

Fig. No.	Sign of picture	Age (year)	Sex	Body height	Cephalic index	Face	Original hair colour	Colour of eyes	Anthropological classification
1.	a	31	male	161,0	85,3	bread	blackbrown	brown	Alpine
	b	36	female	152,4	89,1	very broad	blackbrown	light brown	Alpine + other
2.	a	68	male	—	85,1	medium-broad	brown	green	Armenoid
	b	41	male	159,4	85,1	medium-broad	black	light blue	Armenoid
	c	63	female	149,1	90,9	broad	fair	blue	Armenoid
3.	a	42	male	170,8	85,9	broad	black	dark blue	Pamirian + other
	b	31	female	164,3	84,1	medium-broad	blackbrown	brown	Pamirian + other
4.	a	68	male	157.4	87,8	broad	blackbrown	green	Lappid
	b	41	female	152,3	82,8	very broad	black	blue	Lappid
5.		40	male	168,5	84,9	medium-broad	blackbrown	dark blue	Dinaric
6.		40	male	166,5	84,3	very broad	blackbrown	green	Cromagnoid—C
7.		43	female	158,0	87,5	very broad	blackbrown	grey	Cromagnoid—B + other
8.		35	male	177,0	85,1	broad	brown	dark blue	Cromagnoid—A
9.	a	51	male	156,7	79,9	narrow	blackbrown	brown	gracile Mediterranean
	b	30	female	162,3	81,0	medium-broad	brown	brown	Atlanto-Mediterranean
10.	a	28	female	160,7	83,0	very broad	blackbrown	dark blue	Cromagnoid—C + Mongoloid (Uralian)
	b	50	female	156,5	85,6	very broad	black	dark blue	Turanid + Cromagnoid—C

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